FORMULATION DEVELOPMENT AND EVALUATION OF TRANSDERMAL PATCH CONTAINING AMLODIPINE BESYLATE

Dissertation submitted to

THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY, CHENNAI

in partial fulfillment of the requirements

for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

DEPARTMENT OF PHARMACEUTICS

PERIYAR COLLEGE OF PHARMACEUTICAL SCIENCES FOR GIRLS, TIRUCHIRAPPALLI -620 021.

March 2008

(AN ISO 9001 CERTIFIED INSTITUTION)

Prof.T.N.K.Suriyaprakash M.Pharm.,(Ph.D.)

Head, Department of Pharmaceutics,
Periyar College of Pharmaceutical Sciences for Girls,
Tiruchirapalli-620 021
Tamil Nadu,
India.

CERTIFICATE

This is to Certify that this dissertation entitled **"FORMULATION DEVELOPMENT AND EVALUATION OF TRANSDERMAL PATCH CONTAINING AMLODIPINE BESYLATE" :**by **Mr Shaikh Imran** for the award of "**Master Of Pharmacy"** degree, comprises of the bonafide work done by him in the Department of Pharmaceutics, Periyar College of Pharmaceutical Sciences for Girls, Tiruchirapalli, under my supervision and guidance and to my full satisfaction.

Place: Tiruchirapalli

Date: **(Prof.T.N.K.Suriyaprakash)**

Dr. R.Senthamarai, M.Pharm., Ph.D.,
Principal,
Periyar College of Pharmaceutical Sciences for Girls,
Tiruchirapalli-620 021
Tamil Nadu,
India.

CERTIFICATE

This is to Certify that this dissertation entitled **"FORMULATION DEVELOPMENT AND EVALUATION OF TRANSDERMAL PATCH CONTAINING AMLODIPINE BESYLATE",** by **Mr Shaikh Imran** for the award of "**Master of Pharmacy"** degree, comprises of the bonafide work done by him in the Department of Pharmaceutics, Periyar College of Pharmaceutical Sciences for Girls, Tiruchirapalli, his work was supervised by **Prof.T.N.K.Suriyaprakash M.Pharm., (Ph.D**.), Head, Department of Pharmaceutics, Periyar College of Pharmaceutical Sciences for Girls, Tiruchirapalli.

I recommend this research work for acceptance as project for the partial fulfillment of the degree of "**Master of Pharmacy"** of the Department of Pharmaceutics, Periyar College of Pharmaceutical Sciences for Girls, Tiruchirapalli, for the year March 2008.

Place : Tiruchirapalli

Date: (**Dr.R.Senthamarai)**

ACKNOWLEDGEMENT

Though words are seldom sufficient to express gratitude and feelings, it some how gives me an opportunity to thank those who helped me during the tenure of my study. The work of dissertation preparation was a daunting task and a fascinating experience.

I take this opportunity to express my deep sense of gratitude to my guide **Prof.T.N.K.Suriyaprakash M.Pharm, (Ph.D.),** Head, Department of Pharmaceutics, Periyar College of Pharmaceutical Sciences for Girls, Trichy-21, for his guidance, valuable suggestions and liberal encouragement to complete this work successfully entitled **"FORMULATION DEVELOPMENT AND EVALUATION OF TRANSDERMAL PATCH CONTAINING AMLODIPINE BESYLATE",**

It's my privilege and honor to thank **Dr.R.Senthamarai M.Pharm, Ph.D.,** Principal, Periyar College of Pharmaceutical Sciences for Girls, Trichy-21, for providing all the necessary facilities to do this thesis work.

I extend my sincere thanks to our honorable chairman Man**.K.Veeramani, MA.B.L.,** Chancellor, Periyar Maniyammai University and **Mr.Gnana Sebastian** Correspondent, Periyar College of Pharmaceutical Sciences for Girls, Trichy-21, for providing all the facilities.

My warmest thanks to **Dr..S..Karpagam Kumarasundari M.Pharm, Ph.D** Head, Department of Pharmacology, Periyar College of

Pharmaceutical Sciences for Girls, Trichy-21, for his valuable suggestion and help to complete thesis work.

I express my deep sense of gratitude to **Prof.A.M.Ismail M.Pharm, (Ph.D)** Dean(PG), Periyar College of Pharmaceutical Sciences for Girls, Trichy-21, for his suggestion to complete this work.

My sincere and heart felt thanks to **Mrs.K.Reeta Vijaya Rani M.Pharm, (Ph.D), Mr.M.Sakthivel M.Pharm., Ms.N.Pavala Rani M.Pharm., and Mrs.R.Latha Eswari M.Pharm.,** Department of Pharmaceutics, Periyar College of Pharmaceutical Sciences for Girls, Trichy-21,for their valuable suggestion and help to complete thesis work.

Words are insufficient to express my feeling towards my classmates V.Narayanan, B.Senthilnathan, V.Dineshkumar, A.Sivakumar, Eugien Leo Prakash and other my friends who helped me directly and indirectly in the successful completion of this dissertation.

I convey my special thanks **to Mr.K.A.S..Mohammed Shafique M.Pharm.,** for his constant help and suggestion which were of great importance to me to complete this thesis work.

Also express my heartfelt and sincere thanks to all teaching and non-teaching staff members for their timely help.

It's my privilege to express my sincere thanks to **Mrs.K.Tamilselvi,** Librarian, for providing the library facilities and co-operations to complete this work.

Last but not the least I am glad to express my warm gratitude to all my Teacher, Juniors, Friends, and well wishers for their kind co-operation encouragement and help rendered at various stages of this research work.

I express my heartfelt thanks to **my parents, my sisters** for their support and encouragement throughout my course of study.

SHAIKH IMRAN

1. Controlled drug delivery system

1. 1 Introduction

Over the past twenty-five years, as the expense and complication involved in marketing new drug entities have increased with concomitant recognition of the therapeutic advantage of controlled drug delivery system. There are several seasons for the attractiveness of these dosage forms. It is generally recognized that for many disease states, a substantial number of therapeutically effective compounds already exist. Side effects or the necessity to administer the compound in a clinical setting, however, often limits the effectiveness of this drugs.[1]

The goal in designing controlled drug delivery systems is to reduce the frequency of dosing and increase effectiveness of the drug by localization at the site of action .Reducing the dose required (or) providing uniform drug delivery.

Controlled release drug administration means not only prolonged release, but also implies predictability and reproducibility of drug release kinetics. Controlled drug delivery system is the one, which delivers the drug at a predetermined rate, systematically, for a specific period of time .[2]

FIGURE 1 Theoretical Illustration Comparing Blood Drug Concentration profiles Of A Controlled-Release Drug Delivery System And Conventional Dosage Forms Via Various Routes Of Administration.

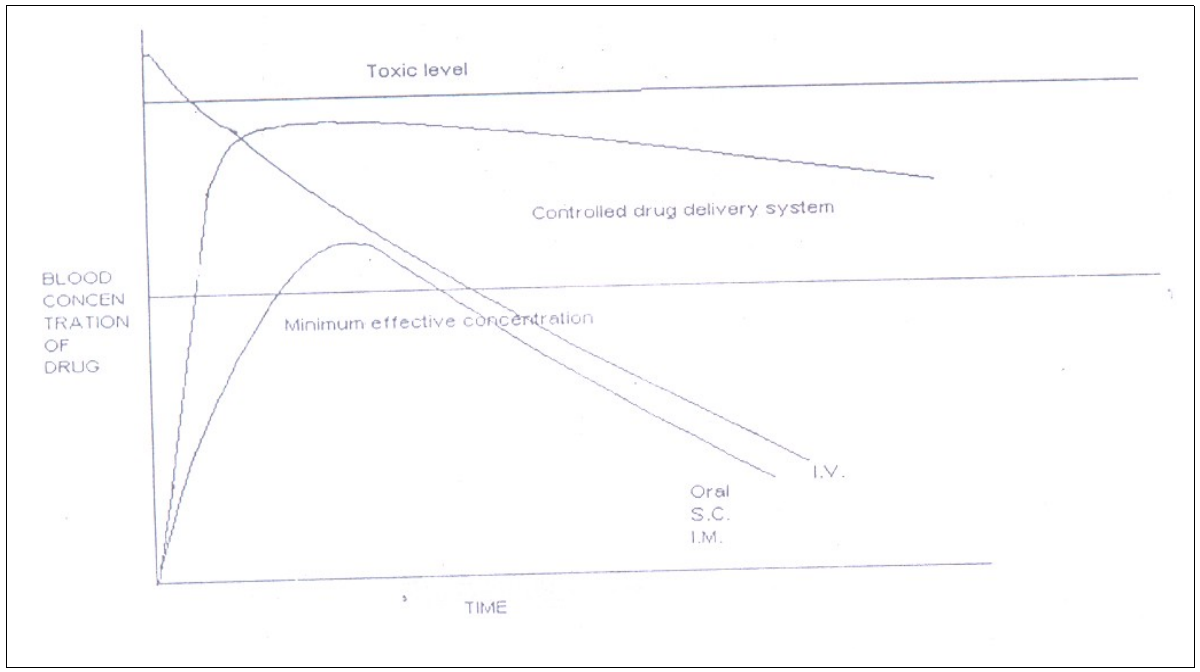


Figure 1 show comparative blood drug level profiles obtained from administration of conventional, controlled as well as prolonged release dosage forms. Thus, the conventional tablet or capsule provides only a single and transient burst of drug. As long as the amount of drug is above the minimum effective concentration, a pharmacological response is observed problems occur when the therapeutic range is very narrow or when the peak is greater than the upper limit of this of this range. Indeed, one of the main purposes of controlled release to improve safety and minimize side effects of the drug by reducing fluctuations in drug level.[3]

MERITS OF CONTROLLED DRUG DELIVERY SYSTEM[4]

1. The potential merit that a controlled release drug delivery system may bring to us can be appreciated by a consideration of prolonged and efficient delivery of therapeutically effective dosages, and localization of therapy.
2. The "peak and valley' pattern is more striking for drugs with biological half-life less than4hrs. Which can be over come by controlled release drug delivery system
3. It provides maximum utilization of drug enabling reduction in total amount of dose administered.
4. Improved patient convenience and compliance due to less frequent drug administration.
5. Reduction in fluctuation in steady state levels and therefore better control of disease condition and reduced intensity of local or systemic side effects.
6. Increased safety margin of high potency drugs due to better control of plasma levels.
7. Reduction in health care cost through improved therapy, shorter time period, less frequency of dosing and reduction in personal time to dispense, administer and monitor patients.

DEMIRITS OF CONTROLLED DRUG DELIVERY SYSTEM[5]

1. Decreased systemic availability in comparison to immediate release conventional dosage forms; this may due to incomplete release, increased first pass metabolism, increased stability, insufficient residence time for complete release, site-specific action, pH-dependant solubility, etc.

2. Possibility of dose dumping due to food, physiologic or formulation variables or chewing or grinding of oral formulation by the patient and thus, increased risk of toxicity.

3. Retrieval of drug is difficult in case of toxicity, poisoning or hypersensitivity reaction.

4. Reduced potential for dosage adjustment of drugs normally administered in varying strengths.

5. It is higher cost of formulation.

1. 2 TRANSDERMAL DRUG DELIVERY SYSTEM

For many decades medication of acute disease as well as a chronic illness has been accomplished dosage forms. Like tablets, capsules, ointments, aerosol, injectables and suppositories, as carrier, recently a technical advancement have resulted in the development of new techniques of drug delivery which includes transdermal drug delivery system. Several transdermal drug delivery systems have been developed to achieve the objective of systemic medication through topical application on the skin surface [4.]

The principle of transdermal drug delivery system is that they could provide controlled drug delivery (have constant drug concentration in plasma) over a prolonged period of time. It is anticipated that transdermal drug delivery system can be designed to input drugs at appropriate rates to maintain suitable plasma drug levels for therapeutic efficacy, without the periodic sojourns into plasma concentration that would accompany toxicity (or) lack of efficacy.

Transdermal delivery of antihypertensive is one of the prime focus areas of drug delivery systems. Various antihypertensive such as metoprolol, clonidine, propranalol, bupranolol, isosorbide dinitrite, verapamil, nifedipine, etc., have been studied for their suitability in transdermal therapeutic systems.

ADVANTAGES OF TRANSDERMAL DRUG DELIVERY SYSTEMS[2]

1. Avoids problems associated with gastro-intestinal absorption due to pH enzymatic activity, and drug food interactions.
2. It is a substitute for oral route.
3. Avoid the risks and inconveniences of I.V therapy.
4. Provides predictable extended duration of activity.
5. Extends the activity of drug with short half-life.
6. Multilayer therapy with single application.
7. Provides capacity to terminate.
8. Minimize inter and intra patient variation.
9. Provides suitability by self-administration.
10. Reduces daily dosing, thus improving patient compliance.
11. Enhance therapeutic efficacy, reduce side effects due to optimization of blood concentration –time profile and elimination of pure entity of drugs into systemic circulation.

LIMITATIONS OF TRANSDERMAL DRUG DELIVERY SYSTEMS[8]

1. Difficulty of permeation through human skin:

- In addition to physical barrier, human skin functions as a chemical barrier. The outer most layer of skin, the stratum corneum is an excellent barrier to all chemicals including drugs. If drug requirements are more than 10mg per day the transdermal delivery will be difficulty. Only relatively potent drugs can be given through this route.

2. **Skin Irritation:**

- Skin irritation or contact dermatitis due to excipients and enhancers of the drug system used for increasing percutaneous absorption is another major limitation.

3. **Clinical Need:**

- It has to be examined carefully before developing a transdermal product.

1.3 TYPES OF TRANSDERMAL DRUG DELIVERY SYSTEMS

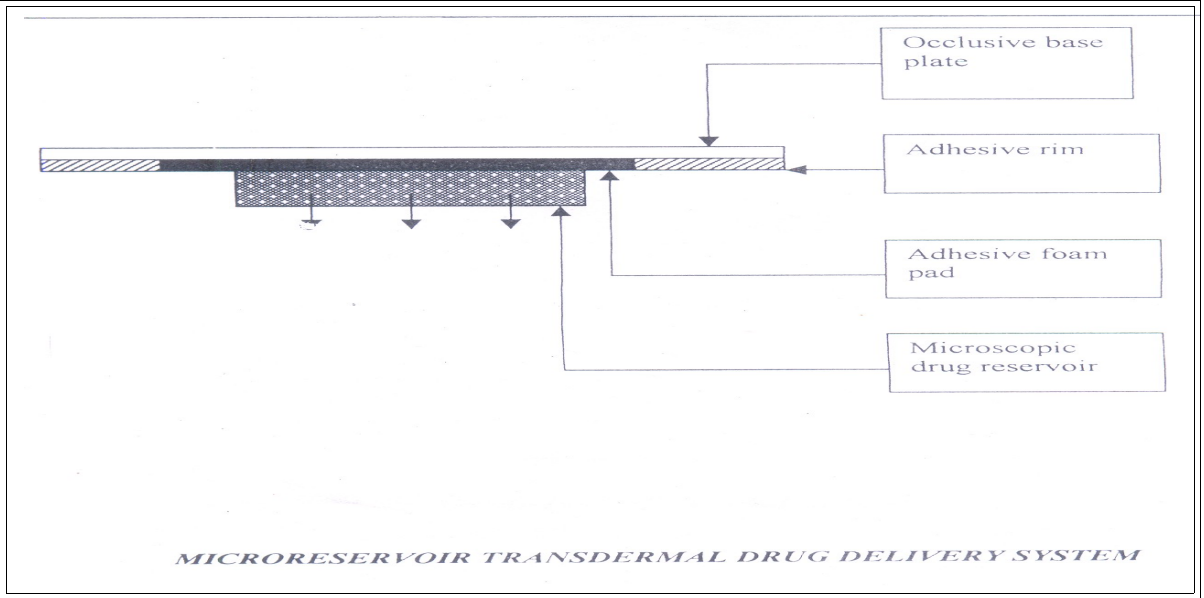
Several approaches can be effectively utilized to control the release of systematically active drugs for permeation at a programmed rate through the skin tissues. The successfully launched commercially available transdermal drug delivery systems may be classified into four types, depending on the technological approach[9].

- Membrane permeation controlled transdermal therapeutic system; (Drugs tried under this are: Scopolamine and Nitroglycerine).
- Adhesive dispersion type transderrmal system. (Drugs tried under this is Nitroglycerine).

- Micro reservoir dissolution controlled transdermal system (Drug tried under this is Nitroglycerin

MICRORESERVOIR TRANSDERMAL DRUG DELIVERY SYSTEM

This is a hybrid of the reservoir and matrix dispersion type drug delivery systems. In this the drug reservoir is formed by first suspending the drug solids in the aqueous solution of a water soluble polymer.(eg. Polyethylene glycol) and then dispersing the drug suspension in a lipophilic polymer, by high shear mechanical force to form thousands of unleachable microscopic spheres of drug reservoir. This is thermodynamically unstable dispersion is quickly stabilized by immediately cross linking the polymer chains in situ. Thus producing a medicated polymer disc with a constant surface area and a thickness.



MICRORESERVOIR TRANSDERMAL DRUG DELIVERY SYSTEM

Figure No.2 Microreservoir Transdermal Drug Delivery System

MATRIX DIFFUSION TRANSDERMAL DRUG DELIVERY SYSTEM

Here, the drug reservoir is formed by homogenously dispersing the drug solids in a hydrophilic or lipophilic polymer matrix, the medicated polymer is then molded into a medicated disc with a defined surface area and controlled thickness. This drug reservoir containing polymer disc is mounted to an occlusive base plate in a compartment fabricated from a drug impermeable plastic backing. The adhesive, in this is spread along with circumference of the patch to form an adhesive rim.

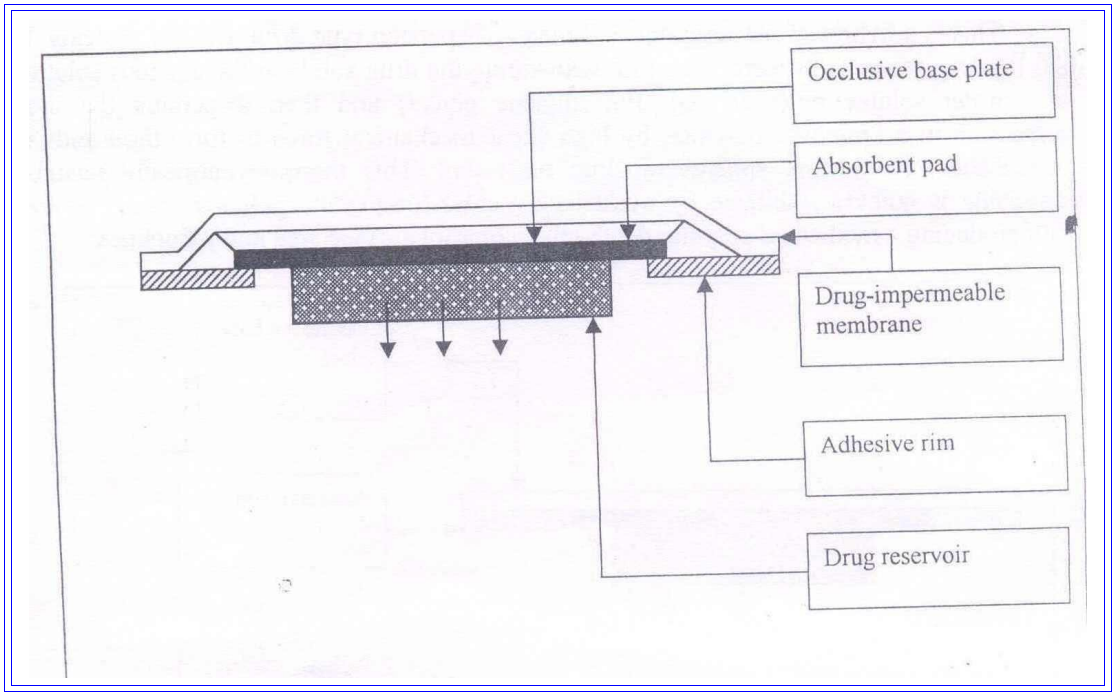


Figure No.3 Matrix Diffusion Transdermal Drug Delivery System

MEMBRANE MODERATED SYSTEM

In membrane moderated systems the drug reservoir is totally encapsulated in a shallow compartment molded from a drug impermeable metallic plastic laminate and a rate controlling polymeric membrane.

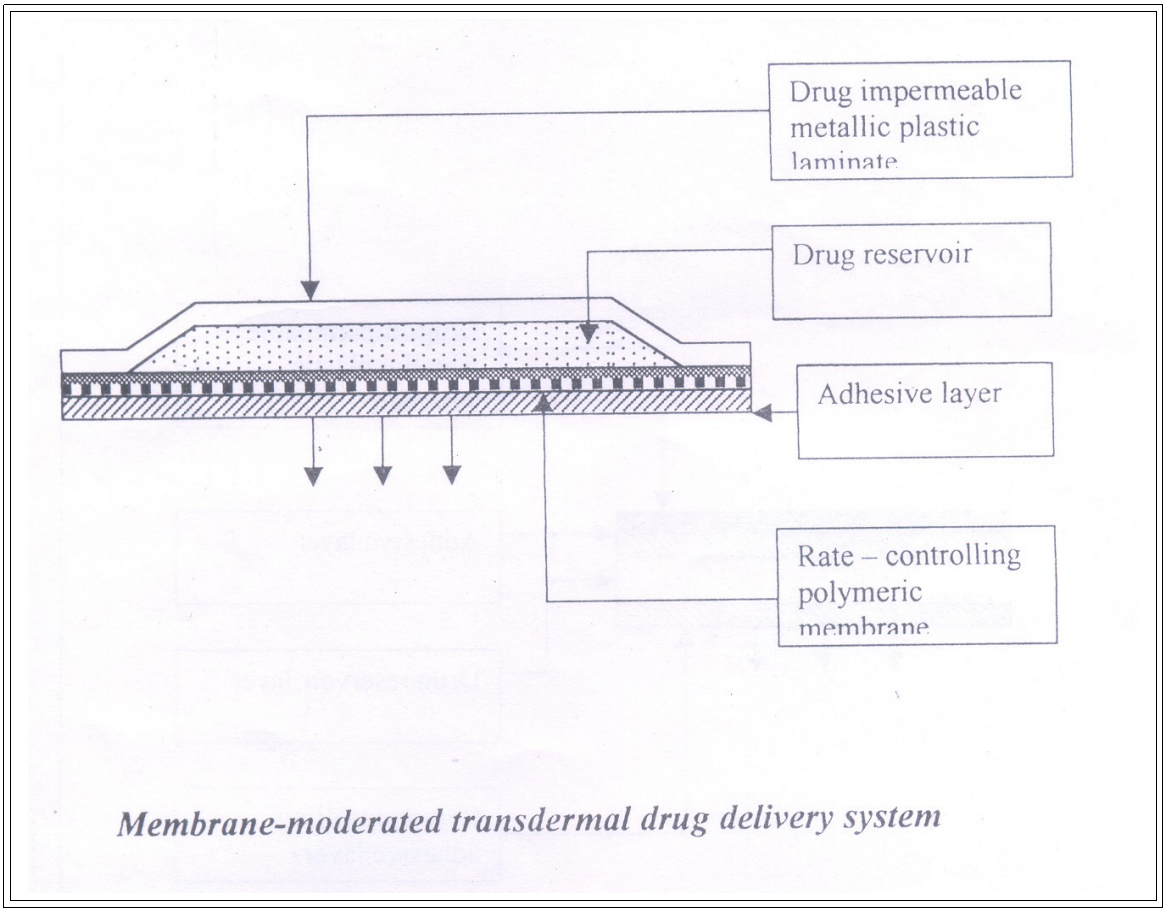


Figure No.4 Membrane Moderated System

ADHESIVE DIFFUSION CONTROLLED SYSTEM:

In this system the drug reservoir is formulated by directly dispersing the drug in an adhesive polymer on a flat sheet of drug impermeable backing to form a thin drug reservoir layer. On the top of the drug reservoir layer, layers of non-mediated rate controlling adhesive polymer of constant thickness are applied to produce an adhesive diffusion-controlled drug delivery system

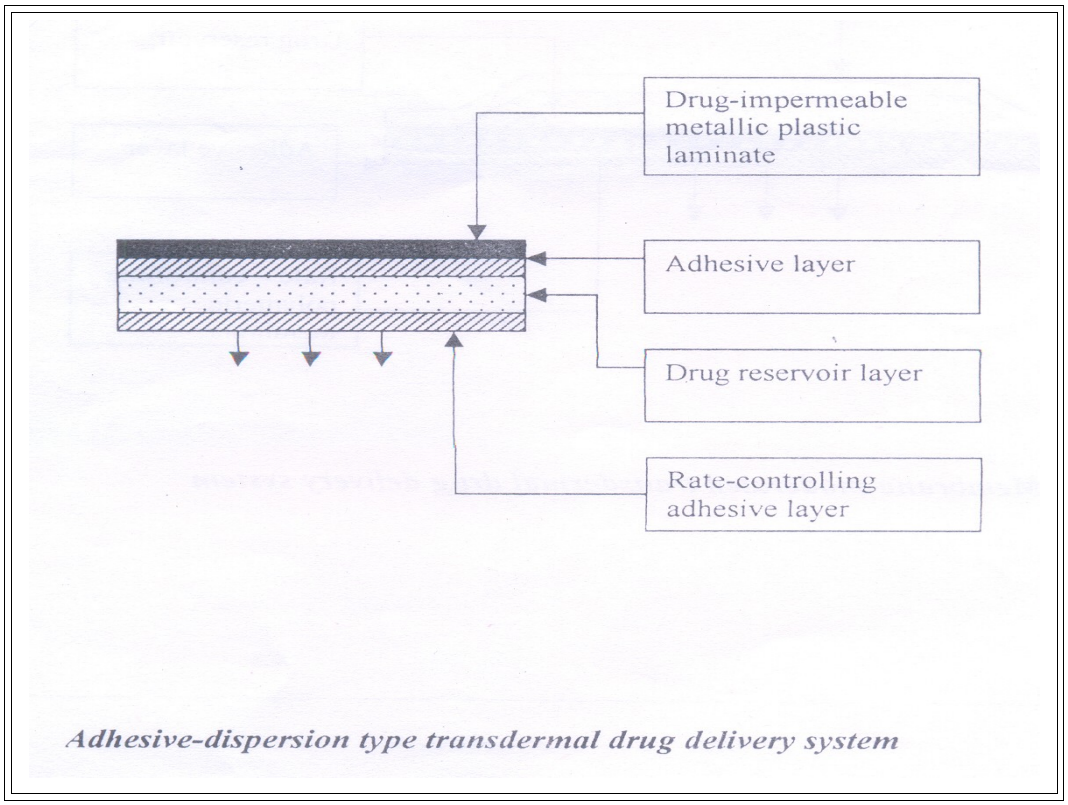


Figure No.5 Adhesive Diffusion Controlled System

Table. No.1 Commercially Available Transdermal Therapeutic Systems

Drug/ Manufacturer	Trade Name	Duration	Type of System	Therapeutic Use
Scopolamine Alza Ciba	Transderm - Scop	2 days	Reservoir	Alleviate motion sickness.
Nitroglycerine Alza/Ciba Hercom Searle Key Wyeth	Transderm – Nitro NTS Nitrodisc Nitro-dur Deponite	1 day	 Reservoir Matrix Matrix Matrix Sandwich	Treatment and prevention of Angina.
Isosorbide dinitrate Nitro electric industrial	Frandol Tape	1 day	Matrix	Treatment and prevention of Angina.
Clonidine Boehringer Ingelheim	Catpres -TTS	7 days	Reservoir	Treatment of hypertension.
Estradiol Ciba-Giegy Parke-davis	Estraderm	3 days	Reservoir	Relief of post-menopausal symptoms.
Nicotine[1] Alza Ciba-Giegy	Nicoderm Habitrol prostep	1 day	Reservoir Matrix Matrix	Aid in smoking cessation.
Fentanyl Janssens	Duagesic	3 days	Reservoir Matrix	Relief from moderate severe pain.
Ketoprofen [2] Pacific Pharmaceuticals	Ketopatch	1 day	Matrix	Analgesic and anti-inflammatory.

1 The product available in India [Transderm – TTS; CIBA-GIEGY; Top nitro; FULFORD(India)].

2. The product is very recently launched in India.

1.4 NOVEL METHODS IN TRANSDERMAL DRUG DELIVERY

Various Methods Have Been Attempted To Enhance Transport

1. Penetration enhancers
2. Iontophoresis
3. Electroporation
4. Sonophoresis
5. Microfabricated microneedles and microchips.

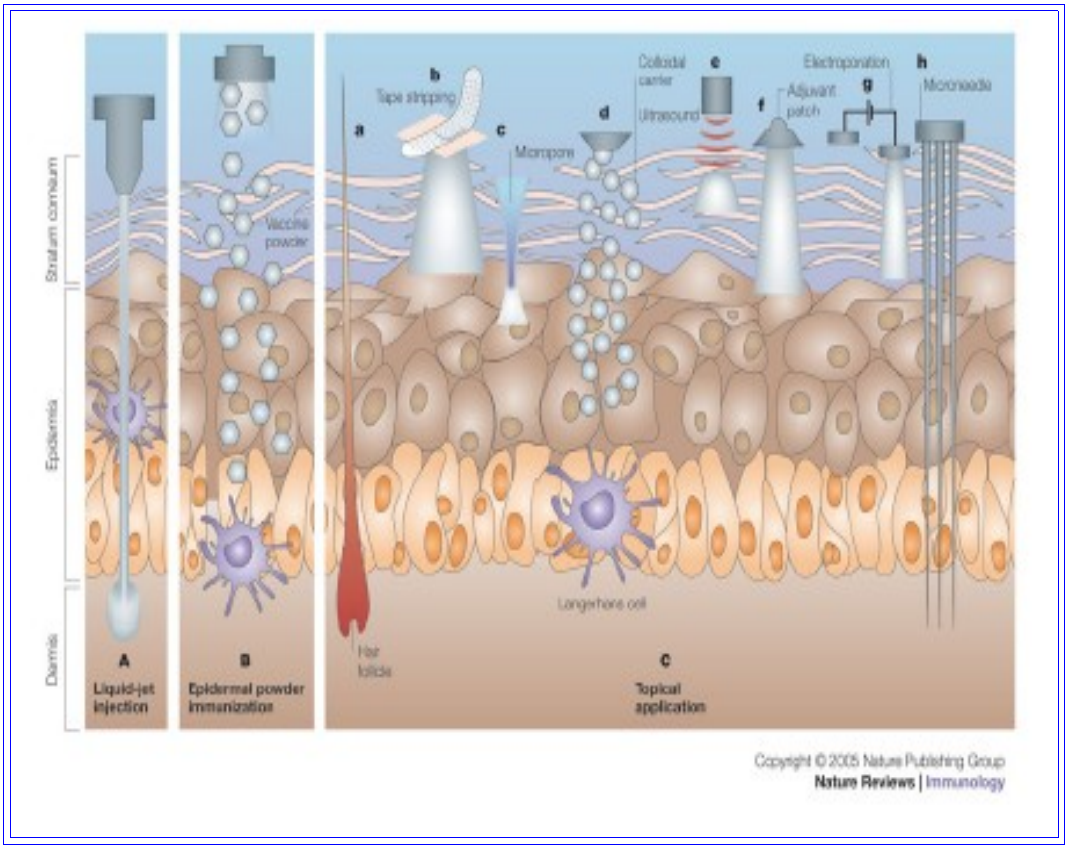


Figure No.6 Various Methods for the TDDS

PENETRATION ENCHANCERS APPROACH

An ideal enhancer should be pharmacologically, inactive, non-irritant and should not damage the skin irreversibly. The effects of an enhancer on the permeation of a drug usually depend upon the physico-chemical characteristics of the permeate as well as the enhancer molecule. The penetration of the enhancers into the stratum corneum is a basic requirement for their efficacy. It is possible to facilitate the penetration of the drug by appropriate pretreatment of the skin with penetration enhancer. The lipoprotein partitioning theory of Barry offers the most acceptable explanation for the skin with penetration enhancers and the stratum corneum. Accordingly, the main reason for enhancement includes:

- Interactions with the intercellular lipids and intracellular keratin.

- Increased penetration of high amounts of enhancer or cosolvent into the stratum corneum for to the improved dissolving capacity of the barrier to the drugs. Many of the chemical enhancers such as dimethyl sulfoxide, surfactants, alcohols, urea, and its derivatives have been screened for their penetration enhacement. The adverse effects caused by some of these enhancers restricts there use widely[11].

IONTOPHORESIS TECHNOLOGY

Iontophoresis is a process or technique involving the transport of ionic or charged molecules into a tissue by the passage of direct or periodic electric current through an electrolyte solution containing the ionic molecules to be delivered using an appropriate electrode polarity. The process involves the transfer of ions into the body by an electromotive force. Ions with positive charge are driven into the skin at the anode and those with the negative charge at the cathode. In the conventional topical treatment by iontophoresis, the drug is administrated through an electrode having the same charge as the drug and a return electrode opposite in charge to the drug is placed at a neutral site on the body surface. The operator then selects a current intensity below the pain threshold level of the patient and allows the current to flow for an appropriate period of time.

The current intensity should be increased slowly, maintained for the length of the treatment and decreased slowly at the end of the treatment. The current must have within comfortable toleration of the patient. A current destiny less than 0.5mA/sq.cm, of the electrode surface has been found to be tolerated by the patient. Interposition of a moist pad between the electrode plate and the skin is necessary for making a perfect contact, preventing any skin burns, overcoming skin resistance and protecting the skin from absorbing a caustic metallic compound formed on the metal plate surface. It is important that, the drug be applied through the electrode with correct polarity may not result in penetration of the drug. The electrode must not come in any direct contact with skin as it may cause burns.

Iontophoresis was found to be widely used in several clinical situations; Iontophoretic delivery of ionizable drugs like, propranolol across excised human skin from rabbit, pig and humans has been reported. Iontophoretic delivery of a weakly basic analgesic, oxycodone across the excised skin from human and several animals was investigated using pulse current delivered from a newly developed transdermal iontotherapeutic system[66] .

ELECTROPORATION TECHNOLOGY

Electroporation or electropermeabilization involves changes in membrane of cell due to application of transmembrane voltages. The change in the membrane involves; structural rearrangement and conductance leading to temporary loss of semipermeability of cell membranes. Suggesting formation of pores. The pulses are normally used on the unilamellar phospholipids bilayers of cell membranes. Approximately 100 multilammeller bilayer of the stratum corneum need about 100V pulses for electroporation or 1V per bilayer. Electroporation of skin takes place at high transdermal voltage (100V or more). There is considerable indirect evidence that high voltage pulses cause changes in the skin structure.

Electroporation is a technique in which the drug encapsulated in vesicles or particles is delivered in to the skin by applying pulse leading to breakdown of the stratum corneum. Pressure medicated electroincorporation has been used to deliver leuprolide acetate micropheres into hairless mouse skin and human skin engrafted on immunodeficient nude mice. It has been shown that application of continuous low voltage resulted in a calcein flux with three orders of magnitude.

Besides, the modes compounds calcein, other drugs investigated for transdermal delivery by electroporation includes; metaprolol flurbiprofen, cyclosporine, heparin, fenanyl and oligonucleotides[67].

SONOPHORESIS TECHNOLOGY[68]

Ultra sound has been used to treat a wide range of clinical condition and to transport drugs to deeper tissues. The movement of drugs through living perturbation is called Sonophoresis. Ultra sound was applied with a sonicator (VCX-400 sonics and materials) operating at a frequency of 20 KHz. The sonicator were operated in the duty-cycle; that is pulsed mode (0.1s on and 0.9s off or 1s on and 9s off or 5s on and 5s off).

Ultra sound may enhance transdermal drug delivery systems by affecting the skin structure (through which enhanced diffusion may occur), by inducing concection or by a combination of both effects. Because, skin conductivity is an excellent indicator of the skin barrier properties.

The ultra sound application has resulted in modest permeation of simple molecular. It was reported that, low frequency ultrasound could be used to deliver insulin across rabbit skin in vivo, resulting in increased plasma hormone levels and lowering of blood glucose.

Measurement of ultrasound intensity: A commonly used calorimetric method was employed to calculate the power from the sonicator based on the change in the temperature of water exposed to the sonicator.

Another method is aluminium foil measurement. In an attempt to quantify the potential of the sonicator to induce transient cavitations in aluminium foil paper. It was mounted onto the diffusion cell in a manner identical to that of the skin sonication was performed for 20s [0.1 on and0.9s off] and then aluminium foil was remove from the cell. The number of pits on the foil was determined by visual inspection. The pits represent physical evidence of the effects of cavitations bubble formation induced by ultrasound.

MICROFABRICATED MICRONEEDLES AND MICROCHIPS TECHNOLOGY[18]

The micro fabricated micro needles technology employs micro-sized needles made from silicon. These micro needle arrays after insertion into the skin create conditions for transport of drug across the stratum corneum. The drug after crossing the stratum corneum diffuses rapidly through deeper tissue and taken up by capillaries for systemic administration. Micro needles were made using the micro fabrication technology similar as that of making of integrated circuits. The micro fabrication technology is simple for cheap and mass production of micron sized structures. For the drug delivery, a three-dimensional array of sharp-tipped micro needles with approximately 150micrometre, in lengths was fabricated. Deep reactive ion etching technique is based on the black silicon method. Each micro needle is about 1 micron in diameter or one hundredth of the diameters of a human hair. These needles can be seen only under a microscope. A microprocessor and pump for delivering tiny amounts of the drug.

The microprocessor and pump automatically inject the right dosage of the drug. The micro needles have extremely sharp tips with radius of curvature less than 1mm facilitating easy piercing into the skin.

1.5 SKIN AS A SITE FOR TRANSDERMAL DRUG DELIVERY SYSTEMS

STRUCTURE OF THE SKIN

The skin consists of different tissues that are joined to perform specific functions. The thickness of the skin cover most of the body is 1-2 mm thick.

Structurally the skin consists of two principal parts. The superficial, thinner portion which is composed of epithelial tissue is the epidermis. The deeper, thicker, connective tissue part is the dermis. Deep to the hypodermis and not part of the skin is the subcutaneous layer. The hypodermis layer consists of areolar and adipose tissues. The sub cutaneous layer serves as a storage depot for fat and contains large blood vessels that supply the skin. This region also contains nerve endings called lamellated corpuses that are sensitive to pressure.

Epidermis

The epidermis is keratinized stratified squamous epithelium. It contains four principal types of cells, keratinocytes, melanocytes, langerhans cells and merkel-cells. About 90% of epidermal cells are keratinocytes which produce the protein keratin[20]

Epidermis consists of following layers:

- Stratum basale,
- Stratum spinosum,
- Stratum granulosum,
- Stratum lucidum,
- Stratum Corneum.

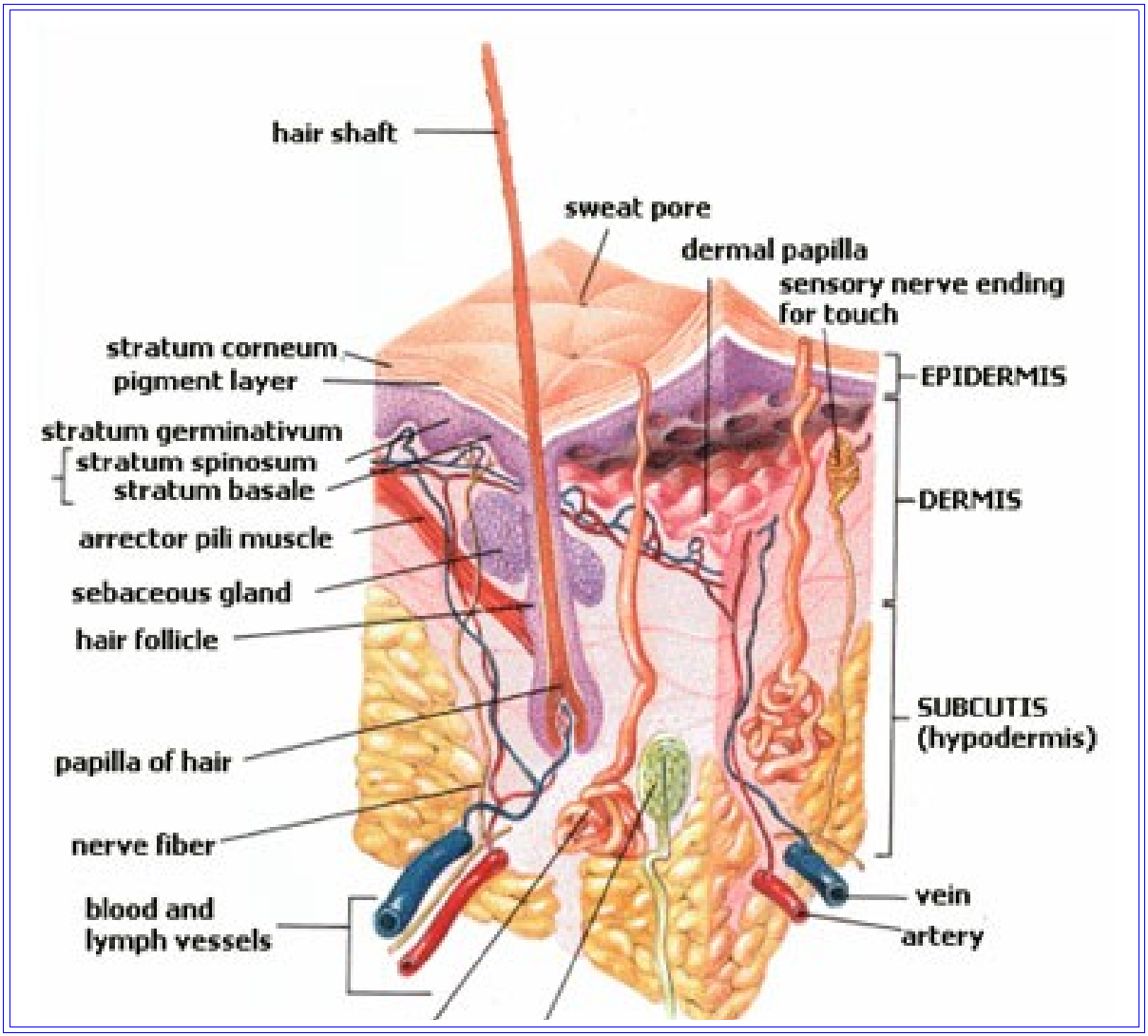


Figure No.7 Structure of the Human Skin

Stratum Basale:

The deepest layer of the epidermis is the stratum basale, composed of a single row of cubical or columnar keratinocytes.

Superficial to the stratum basale is the stratum spinosum where 8-10 layers of polyhedral keratinocytes fit closely together.

Stratum Granulosum:

At the middle of the epidermis the stratum granulosum consists of three to five layers of flattened keratinocytes that are undergoing apoptosis.

Stratum corneum:

The stratum corneum consists of 25-30 layers of dead,flat keratinocytes. The interior of the cells contains mostly densely packed intermediate filaments and keratohyalien. Between the cells are lipids from lamellar granules that help to make this layer water-repellent. These cells are continuously shed and replaced by cells from the deeper strata. The stratum corneum serves as an effective water-repellent barrier and also protects against injury and microbes. Constant exposure of skin to friction stimulates the formation of a callus an abnormal thickening of the epidermis[21].

ROUTES OF PENETRATION

When a molecule reaches intact skin it contacts cellular debris, microorganisms, sebum, and other materials. The diffusing then has three potential entry routes to the viable tissue- through the hair follicles with their associated sebaceous glands, via the sweat ducts, or across the continuous stratum corneum between these appendages. We can summarize relevant

features arriving at a general conclusion[27]

Sebum and surface material

The layer of sebum mixed with sweat, bacteria, and dead cells is this (0.4 – 10 µm), irregular, and discontinuous: it hardly affects percutaneous absorption.

Skin appendages

Their fractional area available for absorption is small (about 0.1%) and this route usually cannot contribute appreciably to the steady state flux. However, the route may be important for ions and large polar molecules which cross-intact stratum corneum with difficulty. Diseases, which disturb the horny layer, such as eczema and exfoliate dermatitis, allow easy access.

Skin appendages may act as shunts, important at short times prior to steady state diffusion, e.g. in bioassays which use pharmacological reactions. Thus minute concentrations of nicotinates or corticosteroids penetration rapidly down the shunt route may trigger erythema or blanching.

Epidermal route

The epidermal layers (particularly the epidermis) may metabolize and inactivate a drug, or activate a prodrug. The dermal papillary layer contains so many capillaries that the average residence time of a drug in the dermis may only be about a minute. Usually, the deeper dermal layers do not influence percutaneous absorption. However, the dermis may bind a hormone such as testosterone, decreasing its systemic removal. If the

penetrant is very lipophililc, it crosses the horny layer to meet an aqueous phase in which it is poorly soluble. The chemical potential immediately below the barrier may then become high, approaching that in the barrier. The potential gradient (stratum corneum to viable tissue) falls, together with the flux. The rate-determining step in percutaneous absorption the becomes barrier clearance not barrier penetration.

Within the stratum corneum, molecules penetrate either intercellular or transcellularly. Electron micrographs of intercellular material suggest a segregation of lipid between protein filaments. In hydrated tissues, these lipid and polar regions would provide parallel pathways for diffusion. Molecules would portion into and diffuse through, either network according to their polarities. The intercellular route is rich in neutral lipid and this pathway may be more important in percutaneous absorption than previously thought.

Topically applied agents such as steroids, hexachlorophane, griseofulvin, sodium fusidate and fusidic acid may form a depot or reservoir by binding within the stratum corneum.

MECHANISM OF SKIN PERMEATION

A systematically active drug that will reach a target tissue from the site of drug administration of the skin surface must posses some physicochemical properties that are capable of facilitating the absorption of drug through the stratum corneum the penetration of drug through viable epidermis and also uptake of the drug by capillary network in the dermal papillary layer. The sequence of transdermal permeation of drug is shown.

The rate of the permeation, dq/dt across the skin tissues can be expressed mathematically by the following relationship.

$$dq/dt = Ps[Cd-Cr].$$

Where

Cd and Cr, respectively the concentration of a skin penetrant in the donor compartment (e.g. Body),

Ps is the overall permeability coefficient of the skin.

$$Ps = Ks\ Dss/hs. \quad \longleftarrow \quad (1)$$

Where

Ks is the partition-coefficient for the interfacial partitioning of a penetrant molecule from the solution of medium or a transdermal drug delivery system on to a stratum corneum;

Dss is the apparent diffusity for the steady state diffusion of the

penetrant molecule through a thickness of skin tissues.[3]

Analysis of (eq-1) suggests that to achieve a constant rate of drug permeation one needs to maintain the drug concentration on the surface of stratum corneum (Cd) consistency and substantially greater than the drug concentration in the body (Cr),

i.e.., Cd>Cr;

Under such a condition eq.1 can be reduced to

$$dq/dt = PsCd$$

The rate of skin permeation dq/dt becomes a constant, if the magnitude of Cd remains fairly constant throughout the course of skin permeation to maintain Cd at a rate (Rd) that is either constant or always greater than the rate of skin update (Ra), i.e. Rd> Ra. By making Rd greater than Ra the drug concentration on the skin surface (Cd) is maintained at a level equal to or greater than the equilibrium (or saturation) solubility of the drug in the stratum corneum (Cs^e).ie., $Cd.C>Cs^e$ and a maximum rate of skin permeation

$$(dq/dt)m = PsCs^e$$

The other mechanism of permeation involves diffusion through shunts particularly those offered by the relatively widely distributed hair follicles and exocrine glands. Typically one square centimeter of human skin yields 10 hair follicles, 15 sebaceous glands and 100 sweat glands. However the appendages provide a small fractional surface area of approximately 0.1% of the total skin area.

Recent studies indicate the importance of appending in percutaneous absorption. The appendages route may be more significant for ions and large polar molecules, which slowly permeates across the bulk of the intact horny layer. The two potential micro pathways serve the stratum corneum through the transcellular and intercellular route.

The principal pathway taken by the penetrant is decided mainly by diffusant's partition coefficient . Most of the diffusant's permeate by both the routes. The intercellular pathway is considered to provide the principal route and the major barrier to the permeation of the drugs.

1.6 FACTORS AFFECTING TRANSDERMAL PERMEABILITY

Physiological and pathological conditions of the skin .[28]

1. **Skin condition**:

- Intact skin prevents penetration. When the skin is exposed to mustard gas, hydrogen sulphide gas, acids, alkalies or ultra violet radiation, skin turns to porous form which will lead to enhanced drug penetration. Mild burns will increase the penetration whereas severe burns retard.

2. **Skin hydration**:

- Hydration results from water diffusing from underlying epidermal layers or from perspiration accumulation after application of an occlusive vehicle or covering on the surface.

- Under occlusive conditions densely and closely packed cells of the skin are opened up and increase its porosity. Occlusion also reduces the "irreversible" binding capacity of stratum corneum. When the skin undergoes hydration, its resistance and capacitance may change. As the time of hydration increases the low frequency impedance of the excised skin decreases with time. A much less activation energy is required to diffuse through hydrated skin.

3. **Skin Age**:

- Fetal and infant skin appears more permeable than adult skin. The stratum corneum of preterm infants is not well developed and as such provides little barrier to the ingress of substances. So this route of delivery is possible for neonatal therapy, when difficulty is encountered in oral or

4. **Increased blood flow:**

- Due to increased blood flow, a concentration gradient is established which will promote drug absorption.

5. Skin Temperature:

- Raising skin temperature results in an increase in the rate of skin permeation. This may be due to thermal energy required differently solubility of drug in skin tissues. Increased vasodilatation of skin vessels.

6.Regional skin sites:

- Difference in the nature and thickness of the barrier layer of the skin causes variation in permeability.

7. Cutaneous drug metabolism:

- Catabolic enzymes present in the viable epidermis may render a drug inactive by metabolism and thus affect the topical bioavailability of the drug.

8.Species variation:

- Human and animals display wide differences in physical characteristics such as the number of appendage openings per unit area and the thickness of the stratum corneum. The average permeability order is Monkey > Dog >Goat > Rat > Guinea pig > Mouse > Human skin.

Physicochemical properties of the penetrant molecules[29].

I. **Partition coefficient**: A lipid/water partition coefficient of 1 or greater is generally required for optimal transdermal permeability. The partition coefficient of a drug molecule may be altered by chemical modification of its functional groups. Membrane partition coefficient increases exponentially as the length of the lipophillic alkyl chain increases.

II. **pH conditions** : Application of solutions whose pH values are very high or very low can be destructive to the skin. With moderate pH values, the flux of ionizable drugs can be affected by changes in pH that alter the ratio of charged to uncharged species and their transdermal permeability.

III. **Drug concentration:** The amount of drug percutaneously absorbed per unit surface area over time interval increase as the concentration of the drug in the vehicle is increased.

IV. **Molecular characteristics of drug:** An inverse relationship appears to exist between absorption rate and molecular weight. Small molecules penetrate more rapidly than large molecules. Drugs with molecular weights of upto 500 Dalton can penetrate well. Drugs with molecular weights above 500 Dalton can be delivered transversally by iontophoresis.

Physicochemical properties of drug delivery systems[30].

I. **Vehicle**: Vehicles serve as drug carriers. Lipophillic solvents facilitate penetration. The absorption of water-soluble and lipid soluble substances from terpene and terpene derivatives was better than from alcoholic solutions. Solubility of drug in the vehicle determines the rate.

II. **Composition of drug delivery systems**: It affects not only the rate of drug release but also the permeability of stratum corneum by means of hydration mixing with skin lipids or other sorption promoting effects.

III. **Enhancement of transdermal permeation**: Transdermal permeation can be increased by including some penetrates, sorption promoters etc. Organic solvents like Dimethyl acetamide, dimethyl formamide, dimethyl sulfoxide, ethylene glycol, propylene glycol and polyethylene glycol.

IV. **Surfactants**: Anionic surfactants are the most effective. E.g. Sodium lauryl sulphate. Chemical like Azone also promote absorption.

1.7 POLYMERS OF TRANSDERMAL DELIVERY SYSTEM

POLYMERS:

The polymer controls the release of the drug from the devices. The following criteria should be satisfied for a polymer to be used in a transdermal system..[31]

1. Drug solubility and diffusivity in the polymer.
2. The desired drug loading and its effect on polymer integrity.
3. Compatibility of the polymer with necessary excepients, such as solvents and skin permeation enhancer of the drug.
4. Skin compatibility: The effect of moisture occluded under the polymer formulation.
5. Mechanical properties: Softness, Flexibility Compatibility to skin and mechanical integrity.
6. Ease of fabrication.
7. Toxicity and purity i.e., compliance with safety requirements of the FDA.
8. Cost and availability.

It is rare to find a commercial polymer that satisfies all the above criteria for polymer selection. Hence various techniques have been

employed to modify the polymer properties and thus drug release rates.

Cross-linked polymers :

The higher the degree of cross-linking the more dense the polymer and slower the diffusion of drug molecule through the matrix cross-linking may be achieved chemically using cross-linking agent or by irradiation. This approached has been applied to the preparation of the Nitrodisc system.

Polymer blend:

The blended polymer combines the advantages of individual polymers. The potential advantages include easy fabrication of devices manipulation of drug loading and other device properties such as hydration, degradation and mechanical strength.

Plasticizers:

Plasticizers are used to reduce the stiffness of the polymer backbone there by increasing the diffusion characteristics of the dug. In selection of plasticizer care must be taken to select a material, which is biocompatible. Commonly used plasticizers are polyethylene glycol, polypropylene glycol, glycerol, and dibutylphthalate and dioctyl phthalate[32].

COMMONLY USED POLYMERS IN TRANSDERMAL FILM

Poly-isobutylene

Poly-isobutylene (PIB) is a highly paraffinic, non-polar and amorphous hydrocarbon polymer composed of essentially straight chain macromolecules. Physical properties of PIB change gradually with increasing molecular weight, the lowest molecular weight polymers being viscous liquids. With increase in molecular weight the liquids are become more viscous, then change to balsam like sticky masses and finally form electrometric solids PIB is soluble in hydrocarbon solvents and insoluble in polar solvents, PIB exhibits excellent low-transition temperature flexibility and oxidative stability.

Polyvinylpyrolidone/Polyvinyl alcohol

Polyvinylpyrolidone (PVP) is a white, odorless and hygroscopic powder. It is available in different viscosity grades, identified by K value. It is soluble in water and in many organic solvents. Polyvinyl alcohol (PVA) is a cream coloured granular powder and is prepared from Polyvinyl acetates. PVA is available in different grades and the viscosity is directly proportional to its molecular weight. Both PVA&PVP are non-toxic to skin and incompatible with inorganic salts.

Ethylene vinyl acetate (EVA) copolymers

EVA'S are ideally suited for preparation of molecular diffusion type membranes because their permeability properties can be varied over a wide range by changing vinyl acetate content. The stiffness, tensile strength and softening point decrease with increasing vinyl acetate content while the permeability and toughness increase. EVA has been shown to be chemically

stable, non-toxic and biocompatible.

Vinyl chloride polymers and copolymers

Vinyl chloride, polymers and copolymers useful for drug/polymers matrix preparation include homopolymers vinyl chloride. $CH_2 = CH\text{-}Cl_2$ and copolymers having a high vinyl chloride content. PVC needs plasticization in order to form a soft and flexible film suitable for transdermal patch formulation. The commonly used plasticizers are dioctyl phthalate, epoxidized Soya bean oil and Citric acid esters.

Cellulose derivatives

Many cellulose derivatives are employed for transdermal drug delivery like ethyl cellulose, methylcellulose, cellulose acetate phthalate, cellulose acetate butyrate, hydroxyl propyl methyl cellulose, carboxyl methyl cellulose sodium they are mostly used in the combination with hydrophilic polymers like PVP, PEG etc.,.

1.8 SELECTION OF DRUG CANDIDATES FOR TRANSDERMAL DELIVERY

The choice of drugs to be delivered is almost a difficult one, and careful consideration should be given for selection of suitable drug molecule. The following are some of the desirable properties of a drug for transdermal delivery.

Physico-chemical properties of the drug:

1. The drug should have a molecular weight of less than 500 Daltons

2. The drug should possess balanced lipophilic, hydrophilic characteristics and also has reasonable solubility in both lipid and aqueous phases. The logP value should be in the range 1-3

3. The melting point should be less than 200 ^{0} C

4. Saturated aqueous solutions of the drug should have pH value between 5 and 9.

5. Hydrogen bonding groups should be less than or equal to 2.

Biological properties of drug

1. The biological half-life ($t_{1/2}$) should be less than 5-6 hours.
2. The drug should be potent with a daily systemic dose of less than 20mg.
3. The drug should not stimulate an immune reaction in the skin.
4. The drug must not induce a cutaneous irritant or allergic response.

2. Literature Review

- **J.Y.Park et. al.,**[71] Studied comparative pharmacokinetic and pharmacodynamic characteristics of amlodipine besylate and amlodiple nicotinate in healthy subject and the results shows mean ratio for $AUC_{0-\alpha}$ and Cmax fell within the predetermined equivalent range of 80-125% pharmacodynamics profiles including systolic and diastolic blood pressures and pulses rates exhibited no significant differences between the two formulations

- **Paul W.Stott. et al.,**[72] Done mechanistic study into the enhanced transdermal permeation of a model β-blocker, propranolol, by fatty acid: a melting point depression effect and found that the binary mixture of propranolol and fatty acid, the addition compounds are formed by interaction between the carbonyl group of the β-blocker, to form a salt. The oppositely charged species of the salt have been shown to permeate the human epidermal membrane by an ion – pair mechanism. This is in agreement with the work by Green and Hadgraft (1987) which suggested the formation of ion-pairs between propranolol and fatty acids. Where the process was driven by a pH gradient.

- **D. Monti et.al.,**[73] Conducted study on the comparison of the effect of ultra sound (US) and of chemical enhancers on transdermal permeation of caffeine(CAF) and morphine through hairless mouse skin in vitro, and found that CAF confirm greater effect of low-

frequency US on skin permeation in vitro. The comparison of US and chemical enhancement indicates a slight superiority of the combination oleyl alcohol (OA) / propylene glycol (PG) over low – frequency US. Concerning morphine (MOR), significantly increased transdermal fluxes were produced by both low frequency US and by OA in combination with PG.

- **Rajagopal K. et. al.,**[74] Formulate and evaluate a matrix type transdermal patches of nimesulide by using different polymers alone or in combination, dibutyl phthalate as the plasticizer and aluminum foils as the backing membrane. The studies showed that (2.2) hydroxyl propyl methyl cellulose (HPMC) and ethyl cellulose (EC) combination may be the suitable polymer combination for the development of transdermal drug delivery system of nimesulide

- **Jawahar N. et.al.,**[10] Prepare and evaluate verapamil hydrochloride transdermal films and study the effect of different formulation variables. The drug diffusion through the films followed a pattern close to zero order type. The drug release profile was decreased with increased polymer concentration and film thickness.

- **L.M.A. Nolan et.al.,**[75] Studies Iontophoretic and chemical enhancement of drug delivery part I: across artificial membranes and reported that the delivery of salbutamol from the fatty acid containing systems was substantially enhanced by iontophoresis and the rates were shown to be approximately proportional to the assisting currents. The data clearly indicates the iontophoretic process to be significantly

less efficient in the presence by buffer lons but with the iontophoretic delivery rates being enhanced by the presence of a fatty acid.

- **M.B.Blanco.et.al.,**[76] Transdermal application of bupivacaine – loaded poly (acrylamide (A)- CO- monomethyl itaconate) hydrogels found the skin flux of the drug was between 90 ± 5 and 16 ± 7 mcg/ cm^2 /h depending on the amount of bupivacaine included in the gel and the gel composition. Skin flux increases with the drug load of the gels. Furthermore as more MMI in the gel slower skin flux of the drug due to bupivacaine gel interactions.

- **A.Nokhodchi. et. al.,** [77] Studies the enhancement effect of surfactants on the penetration of lorazepam through rat skin. And concluded that the increase in flux at low enhancer concentrations is normally attributed to the ability of the surfactant molecules to penetrate the skin and increase its permeatility. Reduction in the rate of transport of the drug present in enhancer system beyond 1% w/w is attributed to the ability of the surfactant molecules to form micelles and us normally observed only if interaction between micelle & the drug occurs.

- **Xiaohong Qi et.. al.,** [78] Studies convolution method to predict drug concentration profiles of 2,3,5,6-tetra – methylpyrazine following transdermal application in rabbit from the in-vitro skin permeation data and found that in – vitro skin permeation tests could be useful to

predict in vivo drug absorption profiles following transdermal application.

- **P.Rama Rao. et.al.,** [79] Provide comparative in-vivo evaluation of propranolol hydrochloride after oral and transdermal administration in rabbits. The PK parameters such as maximum plasma concentration (Cmax), t_{max}, MRT Mean residence time) and $AUC_{0-\alpha}$ were significantly ($P< 0.01$) different following transdermal administration compared to oral administration. The $t_{1/2}$ of transdermally delivered PPN was found to be similar to that following oral administration. But sustained activity was observed over a period of 24hrs after transdermal administration compared to oral. The relative bioavailability of PPN was increased about fivefold to six fold after transdermal compare to oral.

- **Sandip B. Tiwari. et.al.,** [80] Done investigation into the potential of iontophoresis facilitated delivery of ketorolac using rat skin. Results found that pretreatment of the skin with D-limonene in ethanol or D-limonene in ethanol + ultra sound significantly enhanced the iontophoretic flux of the drug in comparision to passive flux with or without pretreatment. Trimodality treatment comprising of pretreatment with D-limonene in ethanol+ultrasound in combination followed by iontophoresis was found to be most potent for enhancing the rate of permeation of ketorolac

- **Michal A.Ashburn. et.al.,** [81] Work on pharmacokinetics of transdermal Fentanyl delivered with and without controlled heat and there results suggest controlled heat might be used to significantly shorten the time needed to reach clinically important fentanyl concentrations, controlled heat might be useful to produce rapid increase in serum concentrations for the rapid treatment of breakthrough pain.

- **Jagdish singh.et.al.,**[82] Done work on electronically facilitated transdermal delivery of human parathyroid hormone (1-34) using porcine skin. The flux of hPTH (1-34) with the electroporation pulses of 100 and 300V followed by lontophoresis at 0.2mA/cm^2 was 10 – and 5 –fold higher respectively, in comparison to the flux with corresponding pulses alone.

- **Franziska Grafe,et.al.,**[83] Work on carrier mediated transport of clonidine in human kerationocytes to characterize transport of clonidine into human keratinocytes to characterize transport of clonidine into human keratinocytes and conclude that clonidine us transported into keratinocytes in a pH – dependent manner by a saturable uptake system different from the keratinocyte choline transporter

- **P.N.Kotiyan. et.al.,**[84] Studies electron bean irradiation; a novel technology for the development of transdermal system of lsosorbide dinitrate (ISDN) dissolving in 2-ethylhexylacrylate (EHA)-acrylic

acid (AA) and solution irradiated on a backing membrane at different doses to get transdermal patches. The ISDN-EHA-AA system developed at an irradiation dose of 50KGY showed a higher skin permeation profile as compared to an internationally marketed transdermal matrix system of ISDN.

- **Elvira Escribans. et. al.,** [85] Done assessment of diclofenaec permeation with different formulation using human skin(0.4mm thick)from plastic surgery as a membrane. The results suggest that topical delivery of sodium diclofenac with an absorption enhancer such as a mixture of oleic acid and d-limonene may be an effective medication for both dermal and subdermal Injuries

- **Jorg kreuter et.al.,**[86] Carry studies on crystallization of estradiol containing TDDS determined by isothermal microcalorimetry X-ray diffraction and optical microscopy as it is still a problem to achieve a stable and prolonged constant drug release, to attain high permeation rates across the skin, the concentrations of the drug dissolved have to be high and often create supersaturated, thermodynamically metastable or unstable systems that possess a high tendency to crystallise.

- **M.Jayne Lawrence et.al.,** [87] Done work on formulation of electrically conducting micro emulsion – based organogels. (MBG). From work conclude that MBG not formed with non-ionic surfactant alone, or when used in combination with another non-ionic surfactant

(regardless of oil used). Which is due to inadequate level of water available for by hydration of the surfactant head group.

- **Akirayamanoto et.al.,**[88] Enhanced transdermal delivery of phenylalanyl-glycine by chemical modification with various fatty acids shows the results that the stability and permeability of Phe-Gly were improved by chemical modification with improved by chemical modification with fatty acids and this enchanced permeability of Phe-Gly by the acylation may be attributed to the protection of Phe –Gly from the enzymatic degradation in the skin and the increase in the partition of Phe-Gly to the stratum corneum.

- **K.C. sung, et.al.,** [89] assess the effect of electro oration on transdermal permeation of nalbuphine (NA) and its prodrug. Study demonstrated that electroporation enhance and control transdermal permeation of NA and its prodrugs. The results also indicated that the physicochemical properties of prodrug has significant effects on kinetics as well as mechanisms of transdermal permeation by electroporation.
- **Ramesh panchagnula et.al.,**[90] work on transdermal delivery of zidovudine effect of vehicles on permeation across rat skin and their mechanism of action, to assess to effect of various solvent systems containing water, ethanol, propylene glycol (PG). Studies support that among all the solvent combinations, highest flux and short lag time were archived with ethanol at 66.6% in water and hence is a suitable vehicle for transdermal delivery of AZT.

3. Aim and Objective

Objective of this study

Cardiovascular drugs have relatively low therapeutic indices, which place responsibility on the dosage forms for maintaining the drug blood level within narrow limit .Conventional dosage forms suffer from variations in the absorption and thus leading to wide fluctuation in the plasma drug concentration.

Amlodipine Besylate, is an antihypertensive drug, having calcium channel blocking activity is therapeutically active even at very low doses. E.g.: 5-10mg/day which is one of the essential requirements of a drug candidate for use in transdermal delivery systems.

It also has other suitable properties which include.

1. Low molecular weight.
2. High lipid solubility
3. Extensive first pass metabolism etc.

Transdermal film prepared by various polymers can exhibit good controlled release properties. So, an attempt is done to formulate transdermal film containing Amlodipine Besylate for achieving following goals.

1. To produce steady state plasma concentration
2. To improve stability of Amlodipine in vivo
3. To reduce adverse effect.

4. Plan of Work

1. Identification of drug by spectrophotometric method (IR and UV).

2. Determination of the, partition coefficient and melting point of Amlodipine.

3. Determination of the drug polymer interaction study between the drug (Amlodipine Besylate) and the polymer (HPMC) through differential Scanning calorimetry (DSC)

4. Study on the extent of in vitro permeation of Amlodipine from Transdermal device in the presence of penetration enhancers.

5. Study on the in vivo release pattern using rabbit.

6. To study the skin irritation of transdermal film using rabbit.

7. Stability Studies

5. DRUG PROFILE OF AMILODIPINE BESYLATE

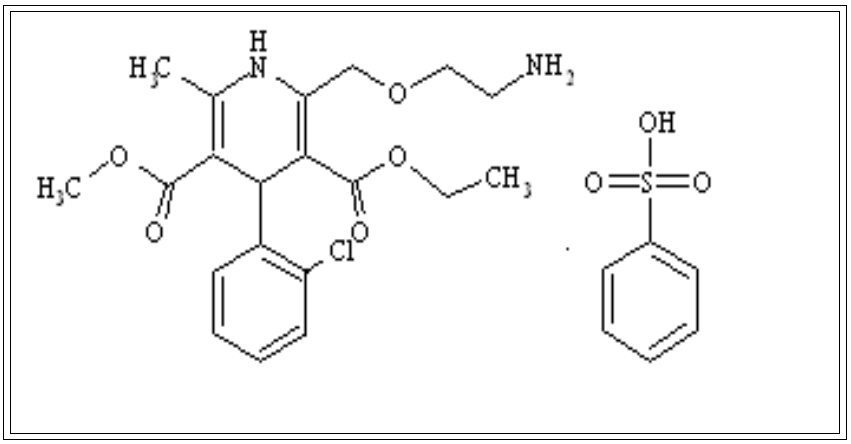
5.1 DRUG PROFILE

Drug Name - Amlodipine besylate

Classification - calcium channel blocker

Synonym - Amlodipine besylate

Chemical structure



Chemical Name - 3-Ethyl 5– Methyl (4R, S) -2- [(2-Amino Ethoxy) Methyl), 4-(2-Chlorophenyl)–6-Methyl–1,4–dihydropyridine–3,5 dicarboxylate benzene Sulphonate

Molecular weight - 567.1

Molecular formula -

Description

Colour - white crystalline powder

Partition coefficient(log P) *-3.00*

Dissociation Constant

(PKa) at 25 ^{0} C - *8.6*

Odour - *odourless*

Solubility - slightly soluble in water

- Sparingly soluble in ethanol

- Freely soluble in methanol

Melting point - 178 -179 ^{0} C

Optical rotation - *Racemic mixture*

CLINICAL PHARMACOLOGY [12, 16]

Amlodipine Besylate is a Dihydro pyridine derivative which is the most potent Ca^{2+} Channel Blockers. Bind to specific sites on the $alpha_1$ subunit, all restricting Ca^{2+} entry

cAMP – phospho diesterase resulting in raised smooth muscle cAMP showing smooth muscle relaxant action.

Released endothelial nitrous oxide may exert anti atherosclerotic action.

Amlodipine inhibits the movement of Calcium Ions (Ca^{2+}) across the cell membrane into vascular smooth and myocytes. Action is greater in the arterial resistant vessels causing peripheral vasodilatation and reduction in after load. Action on the myocardium is considerably less. In angina patient reduction of after load reduces myocardial oxygen requirement.

Amlodipine produces vasodilatation resulting in a reduction of supine and standing blood pressure. Amlodipine does not change sinoatrial (SA) nodal function or atrioventricular (AV) conduction in intact animals or humans. The decrease in blood pressure is not accompanied by a significant change in heart rate or plasma catecholamine levels with chronic dosing. Vasodilatations also increase the oxygen available to the heart in patients with coronary artery spasm and blunt coronary vaso constriction.

PHARMACOKINETICS:

Absorption/ Distribution:

Amlodipine is almost completely well absorbed following oral administration with no effect of food. Bioavailability range is 45 -60% Due to a significant degree of first pass metabolism. Following oral administration, . Time to peak plasma levels occur after 6 -12 hours.

Amlodipine, plasma protein binding is 97.5 % having volume of distribution around 20 L\ Kg. Time to steady state is 7 To 8 days. A normal serum level of Amlodipine may fall in the range 3-11 ng/ml. Amlodipine Crosses the Blood Brain Barrier, Placenta & is excreted in Breast Milk.

Metabolism/ Excretion:

Amlodipine is extensively metabolized in the liver, with 10 % of the parent compound and 60% of the metabolites excreted in the urine. In patients with hepatic dysfunction, decreased clearance of Amlodipine may increase the area under the plasma concentration curve by 40 % - 60 % & dosage reduction may be required. 5% dose of Amoldipine recovered unchanged through urine.

Dosage

Adults: 5 – 10mg once a day

Elderly patient should start with 2-5mg one day & the dose built up as required.

Therapeutic uses:

Amlodipine is used in the treatment for:

Stable and Prinzmetals angina,

Heart failure,

Hypertension.

5.2 Excipients profiles

Hydroxyl propyl methyl cellulose

Non-proprietary Name	Hypromellose
	Hydroxyl propyl methylcellulose
Functional category	Suspending agent
	Coating agents
	Tablet binder
	Film former
Synonyms	Methyl hydroxyl propyl cellulose
	Propylene glycol, ether cellulose
	Hydroxyl propyl methyl cellulose
Chemical Name	Cellulose 2- hydroxyl propyl methyl ether cellulose, hydroxyl propyl methyl

ether.

Typical properties

Density	0.341 gm/cm^3
Solubility	Soluble in cold water forming viscous colloidal solution. Insoluble in alcohol, ether and chloroform.
Grade	HPMC K 100 M HPMC K 4 M HPMC K 15M HPMC k100 LVP
Viscosity	HPMC K 100 M - 8000 to 120 000 m pas HPMC K 4 M - 3000 to 5600 m pas

Application of HPMC in Pharmaceutical Formulation

Film former in tablets film coating. Lower viscosity grades are used in aqueous film coating. Higher viscosity grades may be used to retard the release of drugs from a matrix a levels of 10 - 80 %w/w in tablet and capsule. Depending up on the viscosity grade, concentration of 2 - 20% w/w are used for film forming solution to film coat tablet. Lower viscosity grade are used in aqueous film coating solution, while higher viscosity grades are used with organic solvent.

6.MATERIALS AND METHODS

6.1 Equipments

UV- Visible spectro photometer
Shimadzu – 1700 model.
Open tubular cell
Magnetic stirrer
PH meter
Differential scanning calorimeter : Perkin Elmer, DSC-7
Fourier Transform Infrared : Perkin Elmer. .

6.2 List of reagents

0.1 N Hydrochloric acid

8.5 ml of Concentrated Hydrochloric acid is dissolved in 1000 ml of distilled water.

6.3 Materials

Amlodipine Besylate BP- Standard drug Supplied by Orchid Pharmaceuticals Ltd, Chennai.

Hydroxypropyl methyl Cellulose – Supplied by S.D. Fine chemicals, Bombay.

Methanol – (Analytical grade) supplied by Allied chemicals corporation, Vadodara.

Dimethyl sulphoxides (DMSO)
supplied by Suvidhinath Laboratories, Baroda.

6.4 Methods

Preformulation Studies

1. **IR Determination**

IR determination was carried out by Perkin Elmer Infra-Red Spectrophotometer using KBr Pellet technique [69,70]

2. **Melting point determination**

Melting point of pure Amlodipine Besylate was carried out by open capillary tube method.

3. **Differential scanning calorimetry DSC**[64 70]

The excipient induced modification in the thermal behaviour, were able to correlate long - term stability behaviour simply with the changes in thermal features characteristics of the drug alone. Compatibility and incompatibilities in DSC is concluded by elimination of endothermic peaks

(s), appearance of new peaks (s), change in peak shape and its onset, peak temperature / melting point and relative peak area or enthalpy.

Method

The DSC analysis of each sample under the analogues conditions of temperature range (50 – 55^0C), heating rate (20^0 min^{-1}), chart speed $1cm^{-1}$ under nitrogen atmosphere, with alumina as reference material, were carried out by using Perkin - Elmer DSC -7 analyser.

In order to avoid the need for normalization of enthalpy change of account of great variation in sample weight, care should be taken to conduct DSC analysis accurately. Weight 5 mg or very close to 5 mg portions of the drug, as well as each excipient and transdermal film (2-8mg) equivalent to 5mg of the drug. This would help not only to evaluate data analysis in terms of enthalpy change (loss / gain) associated with the peak of interest but also provide the gross idea about the possibility of interaction simply from visual comparison of the curves relevant to the individual excipient

4. **Determination of partition efficient (P)** [51,57]

Partition coefficient is the ratio of concentration of drug in n-octanol to the concentration of drug in water. The concentration determined by using UV-spectrophotometer.

The intrinsic partition coefficient (P^1) may be more appropriate parameter to measure the partition coefficient of the non-ionized compound. So, P^1 was calculated using formula.

$$P^1 = P\ [\text{antilog}\ [P^k - P^H] + 1$$

6.5 Standard Curve for Amlodipine[61]

Standard curve of Amlodipine were drawn in 0.1NHCl using

UV- Visible spectrophotometer.

Procedure for the Preparation of Standard Curve:-

Amlodipine Besylate 50mg each, were accurately weighed and dissolved separately in 50ml of methanol. Five ml of the above solutions diluted separately to standard stock solution of 100 mcg/ml eight dilutions ranging 5-40 mcg/ml made. The absorbance of each sample was measured using UV-visible spectrophotometer. 0.1 N HCl as blank.The calibration curve was drain by plotting concentration Vs and absorbance to obtain standard calibration curve. The results were shown in table and figure.

Table No.2 Standard Curve for Amlodipine

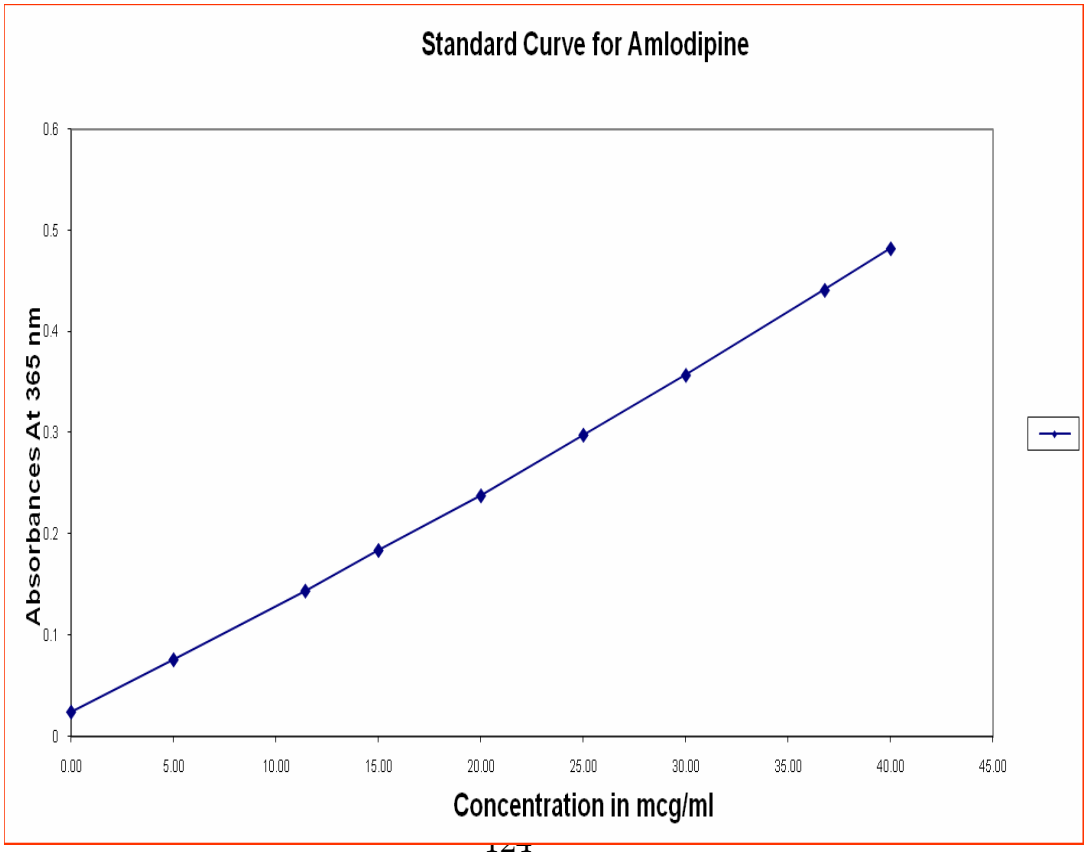
Concentration (mcg/ml)	Absorbance (365nm)
5	0.076
10	0.150
15	0.179
20	0.258
25	0.289
30	0.353
35	0.425
40	0.468

r-0.9968

a-0.0244

b-0.0111

Standard Curve for Amlodipine



6.6 procedure for fabrication of transdermal film

Procedure for fabrication of transdermal. Medicated monolithic films: Transdermal films were prepared by the film casting method of specially designed glass molds with plastic transparent sheets. Different ratio of polymer. 1:4(F2), 1:6(F3) and 1:8(F4) (Drug: HPMC) were used for preparation of films. Varying ratio of polymer dissolved in water. Then drug which dissolved in small quantity of methanol was incorporated in polymer solution obtained by stirring with glass rod for 10 min. The penetration enhancer (Dimethyl sulphoxide) is added to Drug- polymer solution. The solution is poured on petridish using rod. The rate of evaporation of solvent was controlled by inverting cup funnel. After 24 hrs, the dried films were out and stored in desiccator between sheets of paper.

Table No.3 Formula for TDDS

Formulation	Drug (mg)	HPMC (mg)	DMSO (ml)	Solvent (water) (ml)
F1	25	50	2ml	Upto 7 ml
F2	25	100	2ml	Upto 7 ml
F3	25	150	2ml	Upto 7 ml
F4	25	200	2ml	Upto 7 ml

6.7 EVALUATION OF TRANSDERMAL DRUG DELIVERY SYSTEM

Permeation Studies [10,49]

The in vitro permeation studies were carried out using Dialysis membrane. The membrane was prepared using following procedure. The membrane is cut into appropriate size & socked in liquid glycerol for 24 hours for permeable characteristics.

The permeation study was conducted using open tubular cell in the static mode with an effective diffusion area 2.8cm^2. The capacity of receptor compartment was 200ml and temperature was maintained at 37^0c$\pm$1 by means of temperature controller of magnetic stirrers. Drug concentration in donor compartment was 7.473mg/ml Receptor solution was 0.1mHCl which was continuously stirred at 100 rpm with a Teflon – coated bar movement placed inside the cell.

Dialysis membrane was mounted between donor and receptor compartment of the cells and lower side of membrane is in direct contact with the receptor medium. After fixing of patch on membrane, the 5ml of solution withdrawn at specific time interval and equal volume of 0.1N HCl added to receptor solution in an attempt to maintain drug concentration constant through out the experiment (24 hrs). The amount of drug permeated from receptor solution at predetermined times were analyzed by UV-visible spectrophotometer.

In vitro diffusion study[10,62]

The in vitro release profile of Amlodipine from a transdermal device was determined. An open tubular cell was used for the diffusion studies. The dialysis membrane was mounted between donor and receptor compartment of diffusion. The receptor compartment of the diffusion cell was filled with 0.1NHCl. The prepared matrix patch was placed over the membrane. The temperate of the receptor compartment was maintained at 37 ± 1^{0}C. The receptor solution was stirred with Teflon coated magnet stirrer through out the experiment. Aliquots of receptor fluid were withdrawn at predetermined time intervals and replaced immediately with same volume of the fresh fluid, the samples were analyzed at 365 nm using spectrophotometrically.

In vivo release studies[65]

In vivo release studies were carried out using both gender rabbits for transdermal patch containing hydroxy propyl methyl cellulose as polymer and dimethylsulpoxide (DMSO). Four rabbits weighing 1.5 to 2.0kg were selected and the dorsal surface was cleaned and hair was removed. Trans dermal patch of $2.8cm^2$ having dose equivalent to 7.473mg were placed with the help of adhesive tape. Blood samples (0.5ml) were with drawn from the marginal ear (cuboidal vein) into heparinized glass vessels at 0, 0.50, 1, 2, 4, 6, 8, 12, 24 and 48 hrs..The plasma was separated immediately by centrifugation at 2000 rpm for 10min and stored in refrigerator until analysis. The absorbance of the solution was measured at 365nm against a blank

Skin irritation test

A primary skin irritation test was performed since skin is vital organ through which drug is transported. The test was carried out on eight healthy rabbits weighing 1.5 to 2.0 kg. Drug free polymeric films of diameter 2.8cm^2 containing dimethylsulphoxide as a penetrant were used as control. The dorsal surface of rabbits was cleared well and the hair was removed by using a fresh blade. The skin was cleared with rectified spirit. Transdermal patches containing amlodipine (7.47mg equivalent) in HPMC were place over the skin with the help of adhesive tape. The films and patches were removed after 48hrs and the skin was examined for erythema / oedema. All the experimental protocol involving laboratory animals were approved by the IAEC.

6.8 ACCELERATED STABILITY STUDIES [90]

Stability

Stability is officially defined as the time lapse during which the drug product retains the same properties and characteristics that is possessed at the time of manufacture. This process begins at early devolvement phase.

Instability in modern formulation is often detectable only after considerable storage period under normal condition. To assess the stability of a formulated product its usual to expose it to high stress conditions to enhance its detoriation and therefore the time required for testing is reduce common high stress like temperature and humidity. This will eliminate unsatisfactory formulation.

Strategy of stability testing

1. The study of drug decomposition kinetics
2. The development of stability dosage form.
3. Establishment of expiration date for commercially available drug product is some of the needs of stability testing.
4. Data form stability studies should be provided on atleast three primary batches of the drug product.
5. The batches should be manufactured to a minimum of pilot scale.
6. Important point of view of the safety of the patient, patient relieves a uniform dose of drug throughout the shelf life of the product.

Table No.4The Stability Storage condition

S.No	Study	Storage condition	Minimum period
1.	Long-term study	25° C ± 2° C 60 % ± 5% RH	12 month
2.	Intermediate study	30 ° C ± 2° C 60 % ±5 % RH	6 month
3.	Accelerated study	40° C ± 2 ° C 75% ±5 % RH	6 month

ICH (International Conference on Harmonization) Guidelines Specification

1. 5% potency loss from initial assay of batch
2. Any specification degradation that exceed specification
3. Product failing out of pH limit
4. Dissolution out of specification for 12 minutes
5. Failure to meet specification for appearances and physical properties

Any one condition is observed then the stability of the batch is failed.

Procedure

Stability studies were carried out using temperature controlled environment test chamber according to ICH guidelines by storing the formulated films (F_2) at 40^0c / 75 $\pm$ 5% RH for a period of 3 months. The samples were withdrawn at 30, 60 and 90 days and analysed for drug content by spectrophotometrically.

7. Results and Discussion

Preformulation Studies

Drug Identification

Method - Spectrophotometer method (UV)

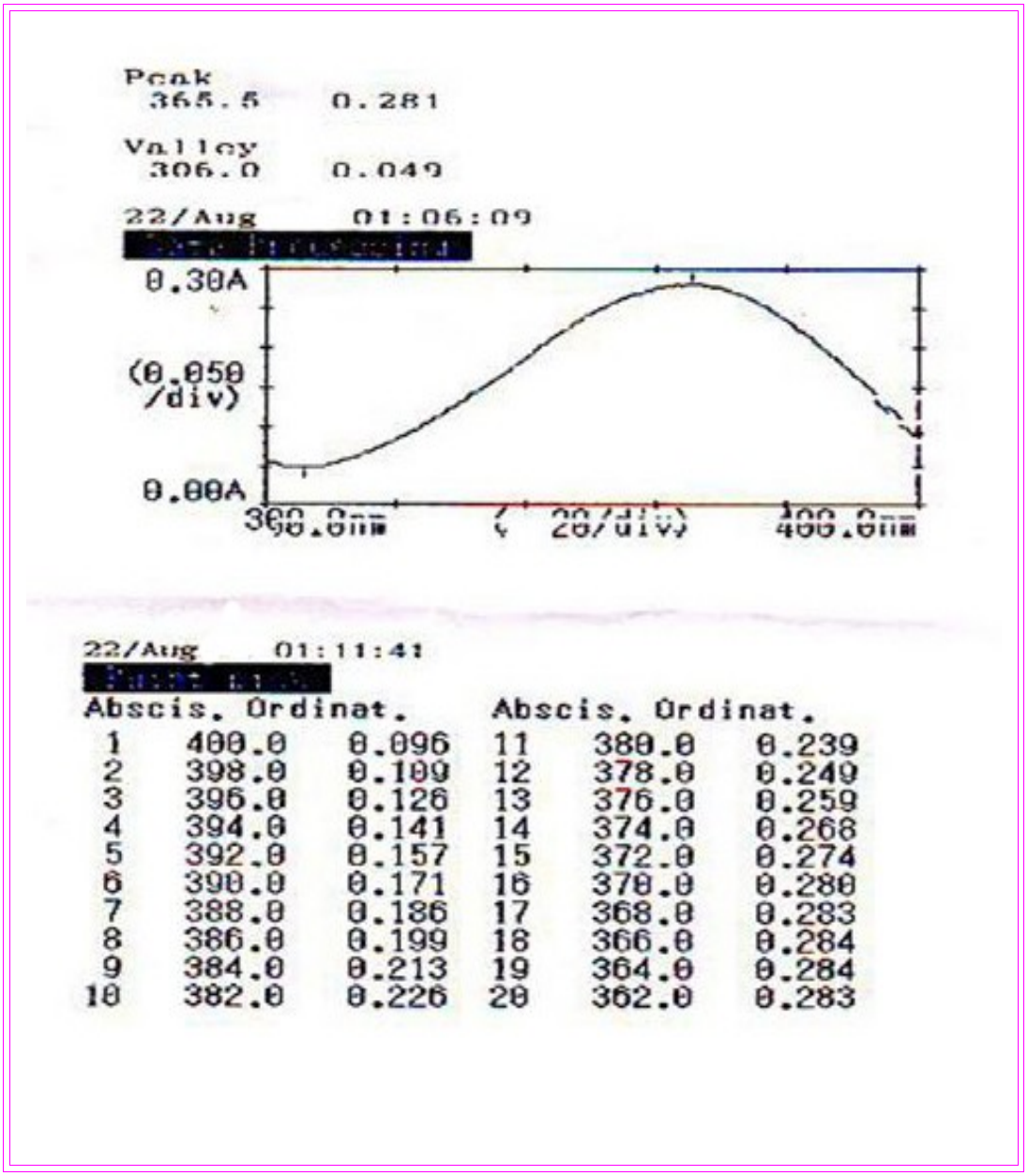
Table No.5 UV- Absorbance of Amlodipine Besylate in 0.1 N HCl

S. No	Concentration in mcg/ml	Absorbance at 365nm
1	25 mcg/ml	0.281

Discussion

The UV- absorbances of the Amlodipine besylate were performed at 25 mcg/ml concentration in 0.1 N HCl and their wavelength were found and compared with monograph.

Figure No.7 UV- Absorbance of Amlodipine Besylate in 0.1 N HCl



22/Aug 01:11:41

Abscis.	Ordinat.		Abscis.	Ordinat.
1	400.0	0.096	11	380.0	0.239
2	398.0	0.109	12	378.0	0.249
3	396.0	0.126	13	376.0	0.259
4	394.0	0.141	14	374.0	0.268
5	392.0	0.157	15	372.0	0.274
6	390.0	0.171	16	370.0	0.280
7	388.0	0.186	17	368.0	0.283
8	386.0	0.199	18	366.0	0.284
9	384.0	0.213	19	364.0	0.284
10	382.0	0.226	20	362.0	0.283

Infra-Red Spectrum

Table No.6 IR Spectrum of Amlodipine Besylate

S.No	Frequency cm-1	Functional Group
1	3411	NH Stretching
2	2813	CH-aliphatic
3	1672	C=0 ester
4	561	C - C1 (C1 vibration)
5	1120	- C- 0 - ester
6	1382	– C - CH3
7	1593	- C = C - aromatic
8	756	Mono substituted aromatic
9	1208	C - 0 Stretching
10	1204	Phenolic Stretching

Discussion

- The structure of Amlodipine Besylate was confirmed by IR spectrum (performed by KBr pellet method).

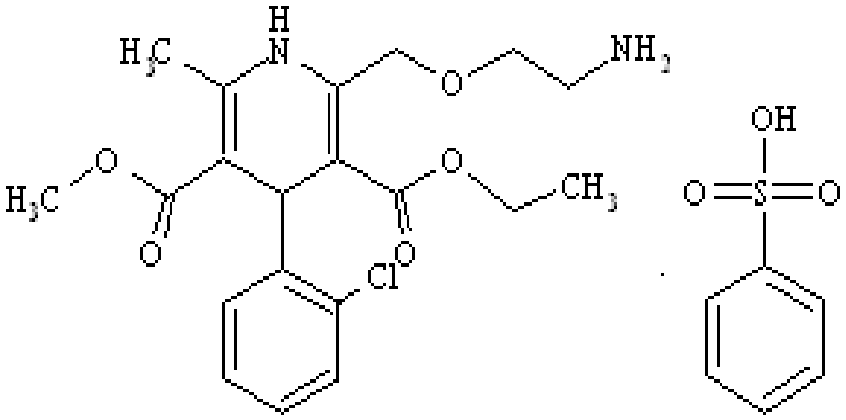
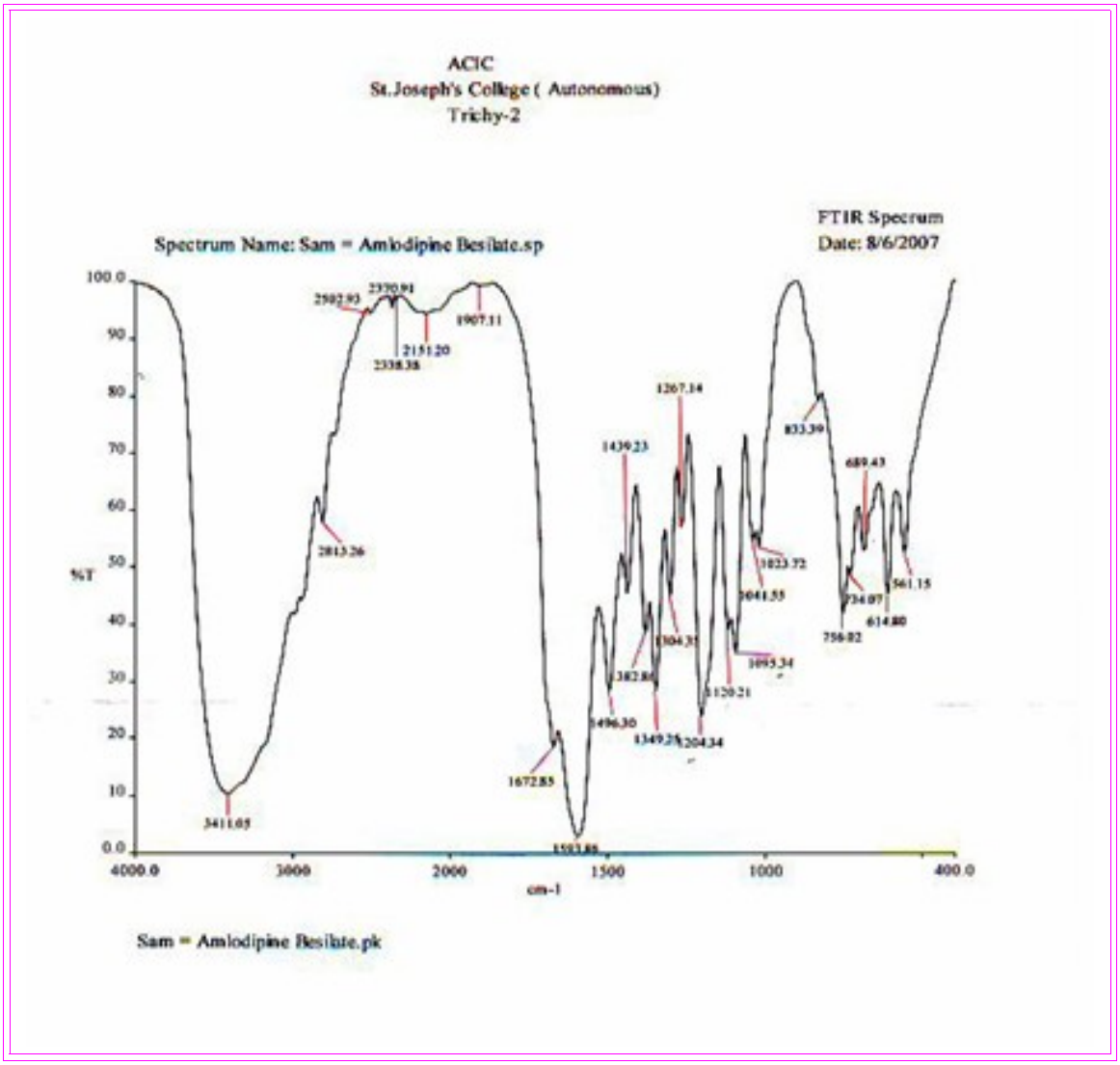


Figure No.8 IR Spectrum of Amlodipine Besylate



Compatibility Studies

DSC Graph for Amlodipine Besylate Patch

Discussion

Differential scanning calorimetry (DSC) was used for compatibility studies between the drug and the excipients. The DSC graph shows that the drug and the excipients are compatible with each other as no elimination of endothermic peak occur and the melting point of drug was also seen in the DSC graph of transdermal film. From above all, the interpretation conclude that the drug and excipients (HPMC) are compatible with each other when they are in the film form

Figure No.9 DSC Graph for Amlodipine

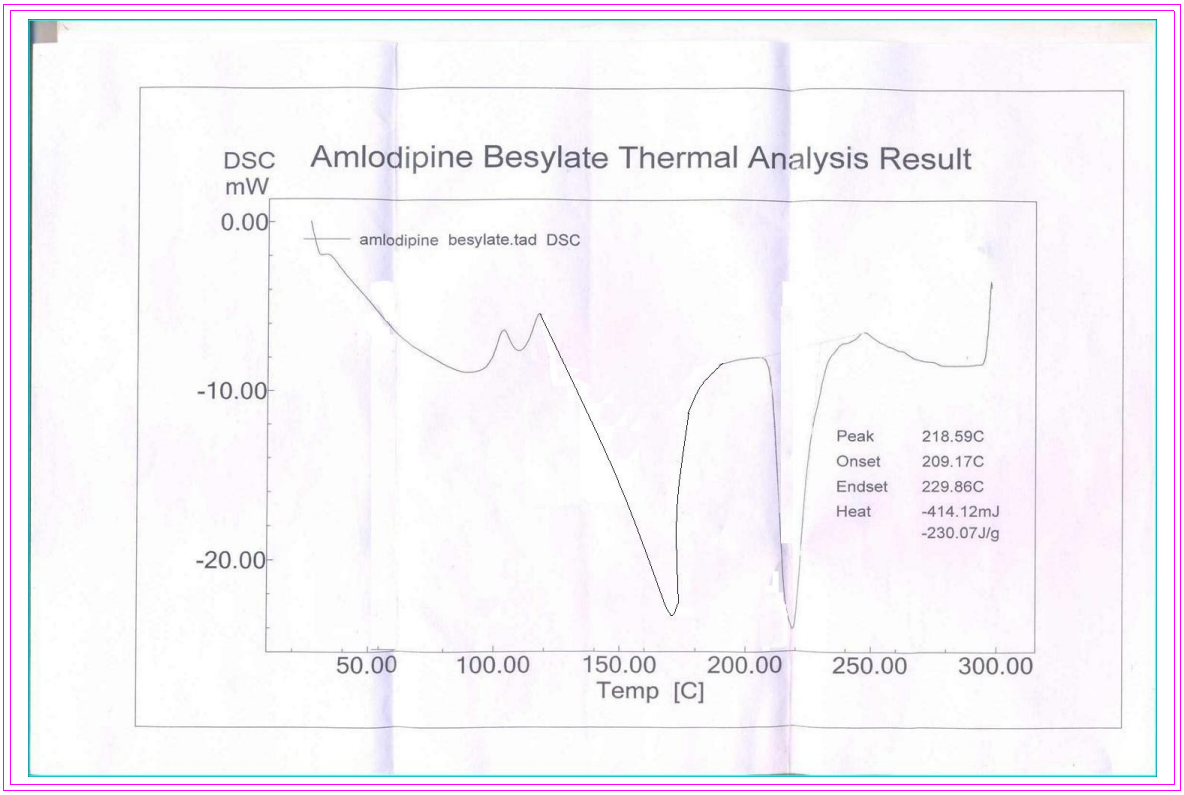


Figure No.10. DSC Graph for HPMC

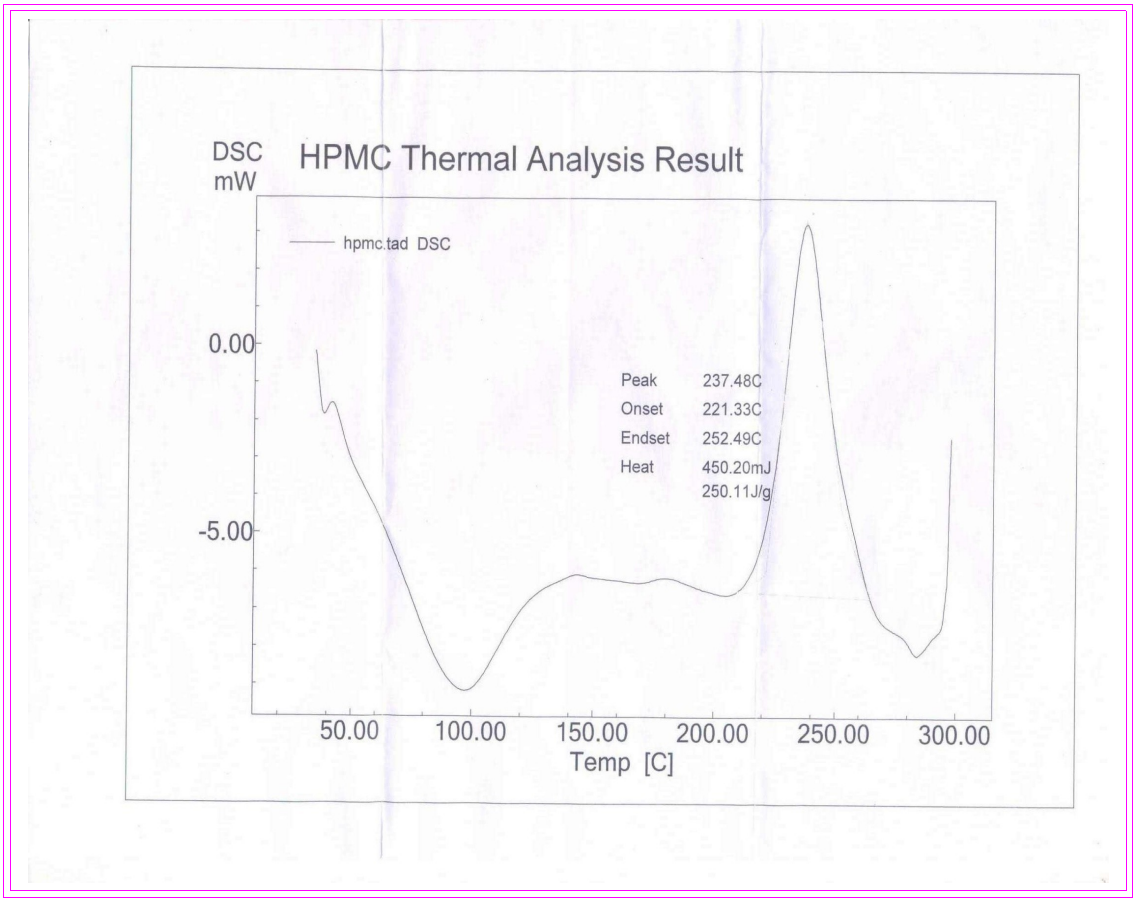
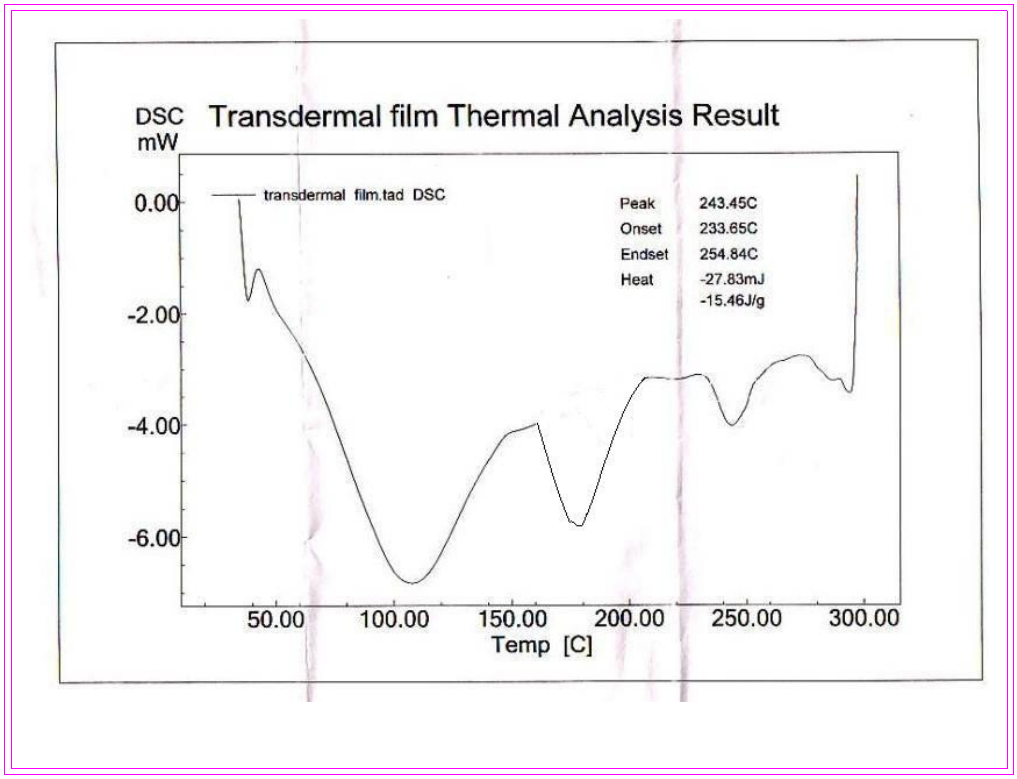


Figure No.11 DSC Graph for Amlodipine Besylate Patch



In vitro Drug Release Profile

Diffusion profile for Amlodipine Besylate in 0.1 N HCl

Table No. 7 In vitro release profile F2 Formulation

S.No	Time in hrs	Absorbance in nm	Concentration in mcg/ml	Amount in mg	% Drug release
01	0	0	0	0	0
02	0.25	0.035	0.954	0.191	2.55
03	0.50	0.046	1.946	0.389	5.20
04	1.00	0.065	3.658	0.732	9.79
05	1.50	0.091	6.055	1.210	16.17
06	2.00	0.121	8.703	1.740	23.28
07	3.00	0.135	9.963	1.992	26.5
08	4.00	0.146	10.954	2.190	29.30
09	6.00	0.157	11.945	2.389	31.96
10	8.00	0.170	13.117	2.623	35.09
11	12.00	0.194	15.279	3.055	40.81
12	16.00	0.220	17.621	3.524	47.10
13	24.00	0.265	21.675	4.335	57.94

Figure No.12 In vitro Drug Release Profile for F2 Formulation

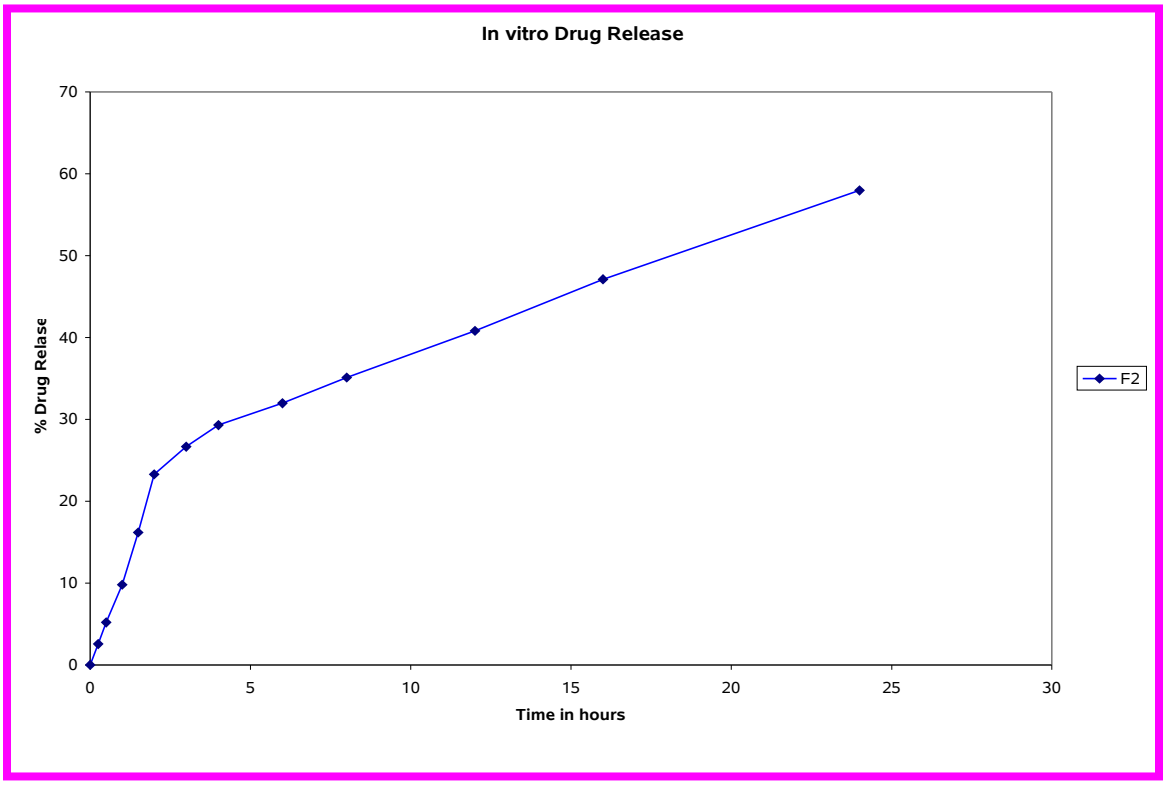
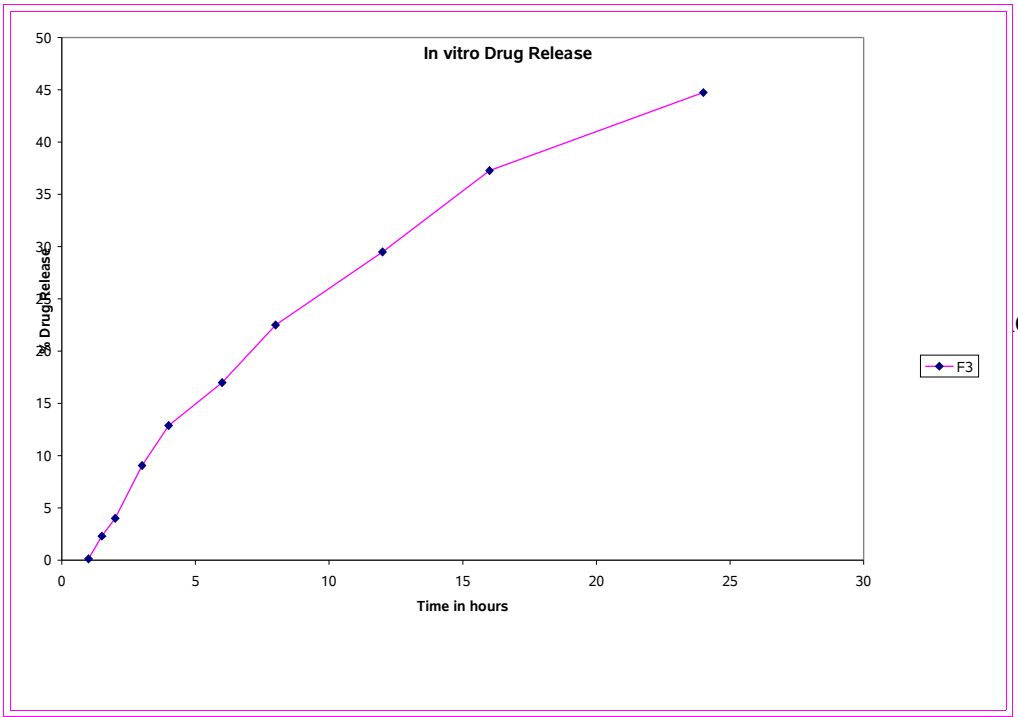

In vitro Drug Release
% Drug Relase
Time in hours
F2
0
10
20
30
40
50
60
70
0
5
10
15
20
25
30

Table No.8 In Vitro Release Profile F3 Formulation

S.No	Time in hrs	Absorbance in nm	Concentration in mcg/ml	Amount in mg	% Drug release
01	0	-	-	-	-
02	0.25	0.008	-	-	-
03	0.50	0.017	-	-	-
04	1.00	0.025	0.054	0.010	0.13
05	1.50	0.034	0.872	0.174	2.32
06	2.00	0.041	1.495	0.299	4.00
07	3.00	0.062	3.387	0.677	9.05
08	4.00	0.078	43828	0.965	12.91
09	6.00	0.095	6.360	1.272	17.02
10	8.00	0.118	8.432	1.686	22.56
11	12.00	0.147	11.045	2.209	29.55
12	16.00	0.179	13.927	2.785	37.26
13	24.00	0.210	16.720	3.344	44.74



on

Table No. 9 In Vitro Release Profile F4 Formulation

S.No	Time in hrs	Absorbance in nm	Concentration in mcg/ml	Amount in mg	% Drug release
01	0	-	-	-	-
02	0.25	0.008	-	-	-
03	0.50	0.012	-	-	-
04	1.00	0.024	-	-	-
05	1.50	0.030	0.504	0.100	1.33
06	2.00	0.041	1.495	0.288	4.00
07	3.00	0.055	2.756	0.551	7.37
08	4.00	0.069	4.018	0.803	10.74
09	6.00	0.080	5.009	1.001	13.39
10	8.00	0.097	6.540	1.308	17.50
11	12.00	0.120	8.612	1.722	23.04
12	16.00	0.154	11.675	2.335	31.24
13	24.00	0.171	13.207	2.641	35.34

Figure No.14 In vitro Drug Release Profile for F4 Formulation

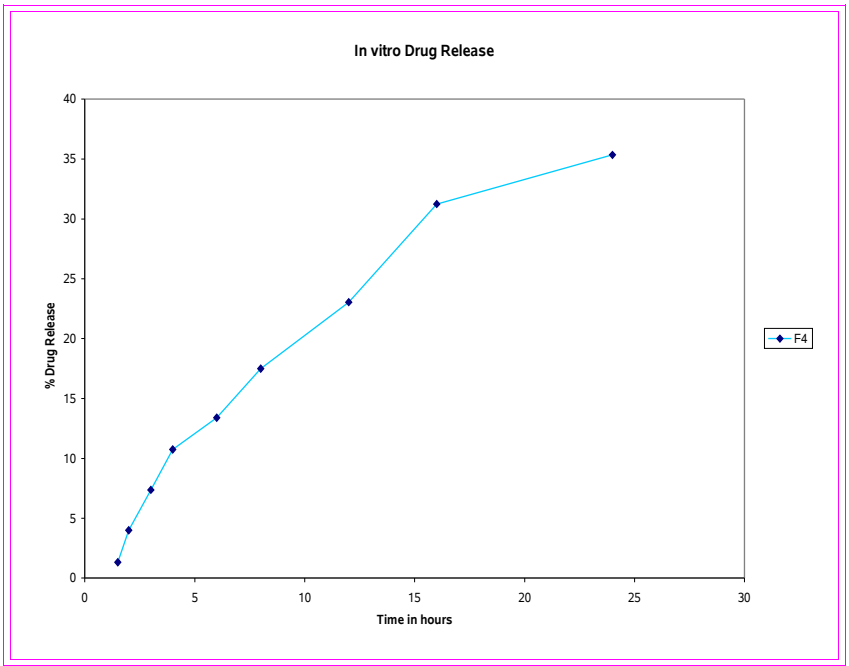
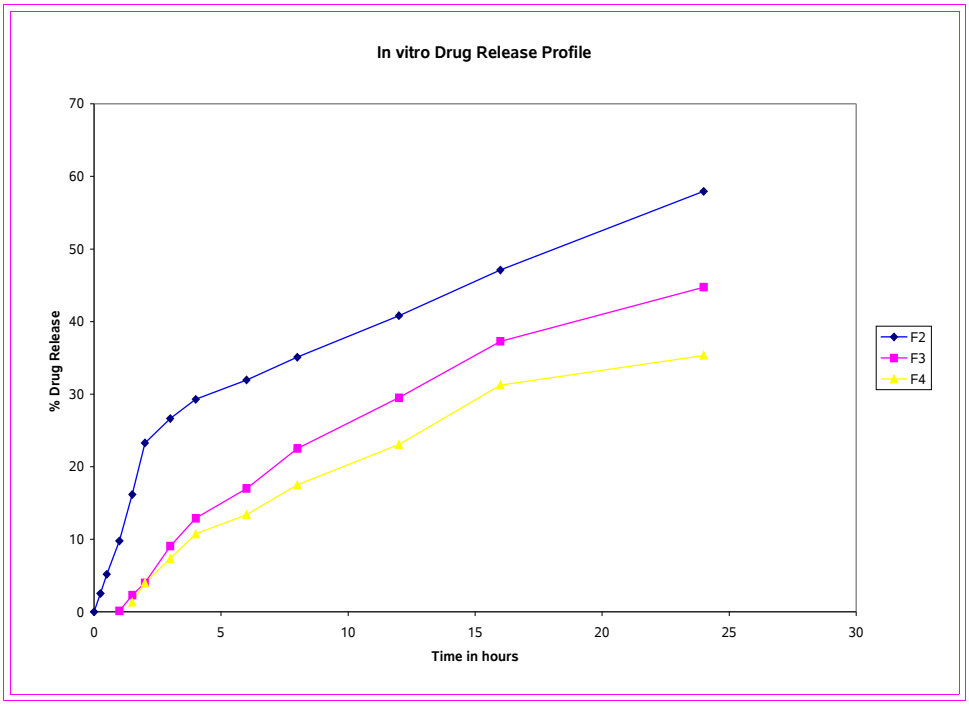


Figure No.15 Comparative In vitro Drug Release Profile



In vivo Drug Release Profile

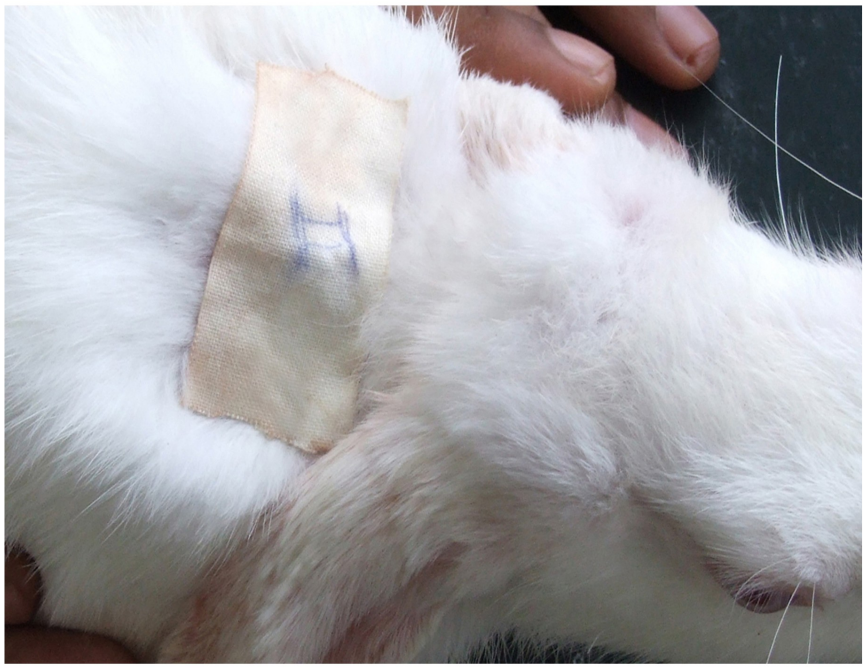
Table No.10 In Vivo Release Profile Control Formulation

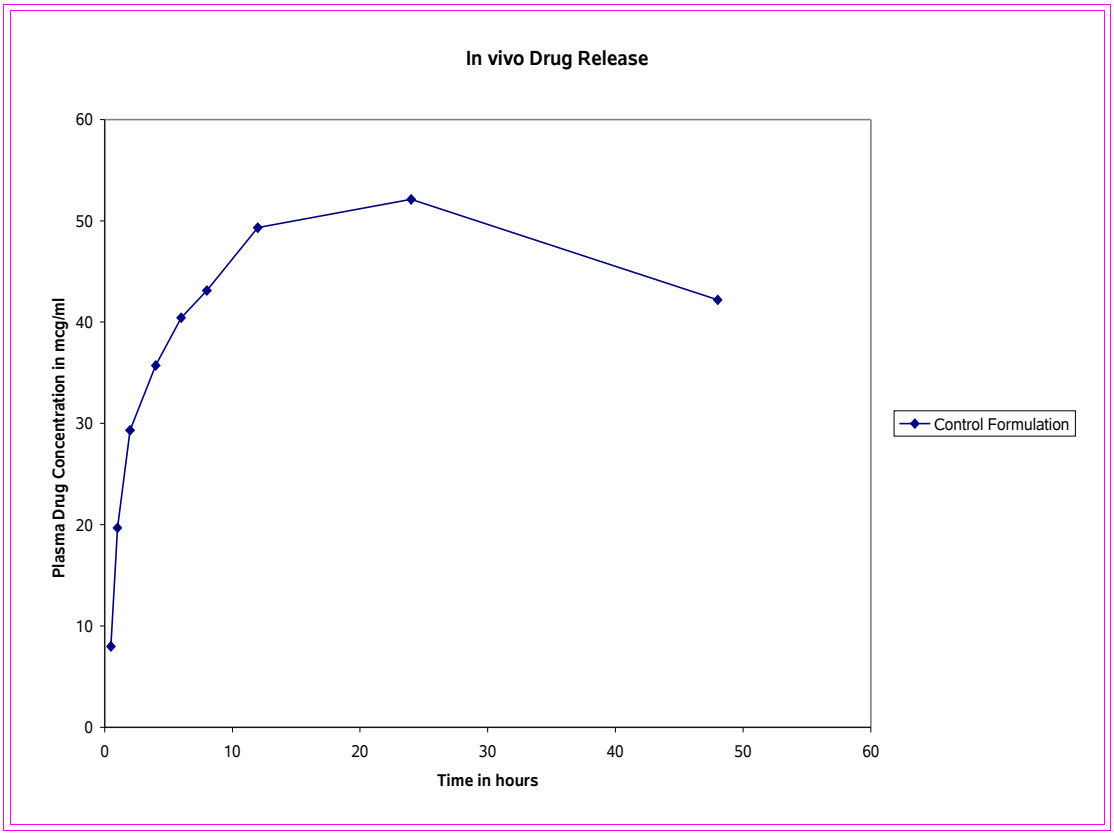
Time in hrs (t)	Absorbance in nm	Plasma drug concentration µg / ml	Amount of drug in mg	% Drug release	C T	$[AUC]^t_0$ µghr/ml	$[AUMC]^t_0$ µghr^2/ml
0	0	-	-	-	-	1.995	0.9976
0.50	0.113	7.981	0.798	7.98	3.990	6.918	5.9208
1.00	0.243	19.693	1.969	19.69	19.693	24.513	39.1765
2.00	0.350	29.333	2.933	29.33	58.666	65.062	201.582
4.00	0.421	35.729	3.572	35.72	142.916	76.143	385.4
6.00	0.473	40.414	4.041	40.41	242.484	83.531	587.42
8.00	0.503	43.117	4.311	43.11	344.936	184.9	1873.864
12.00	0.572	49.333	4.933	49.33	591.996	608.754	11058.12
24.00	0.603	52.126	5.212	52.12	1251.024	1132.104	39328.704
48.00	0.493	42.216	4.221	42.21	2026.368	0	0

∑ AUC = 2183.92

∑ AUMC = 53481.1849

In vivo **Studies**




In vivo Drug Release
Plasma Drug Concentration in mcg/ml
Time in hours
0
10
20
30
40
50
60
Control Formulation

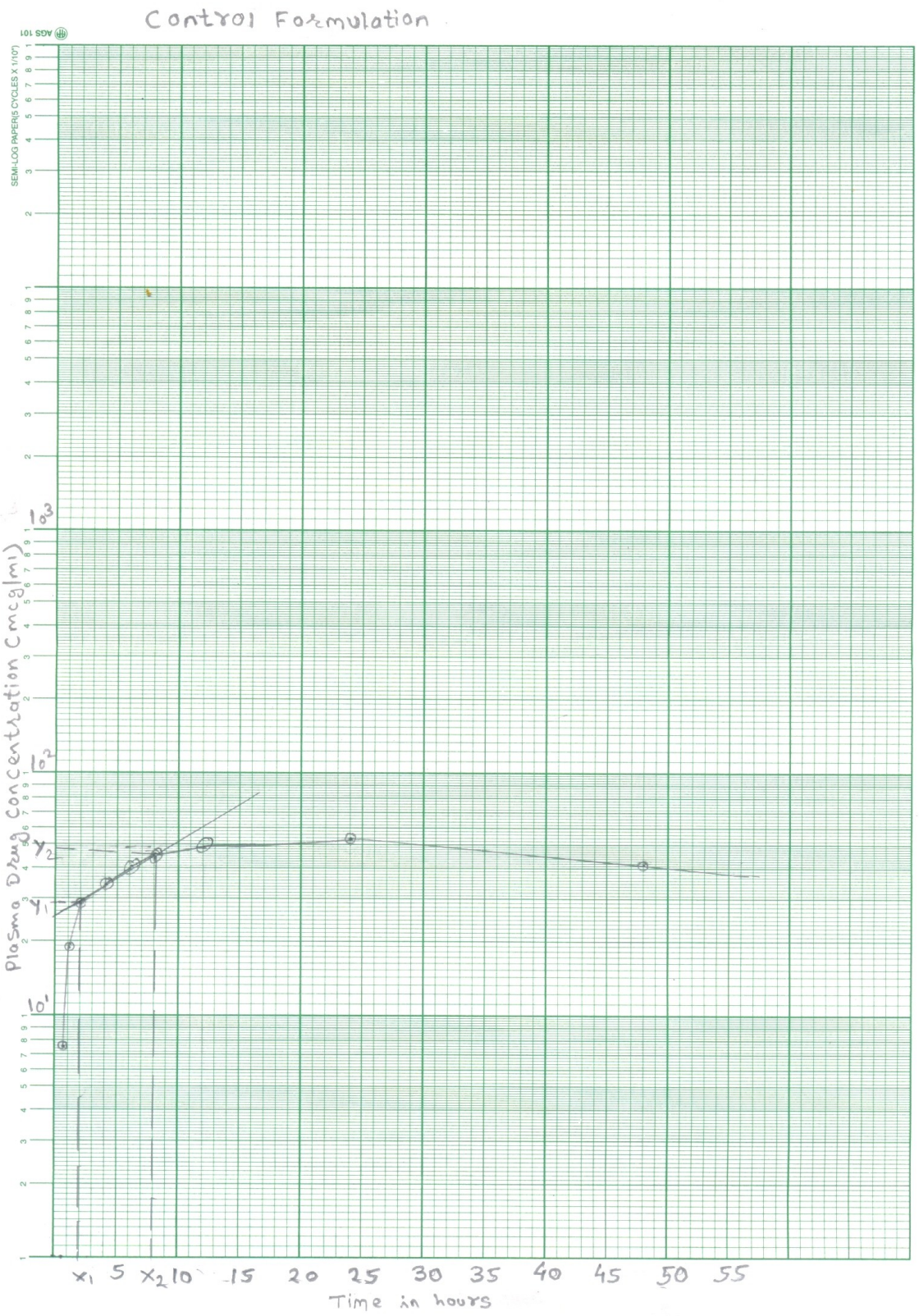

Control Formulation
SEMI-LOG PAPER(5 CYCLES X 1/10")
Plasma Drug Concentration Cmcg(ml)
10^3
10^2
10^1
Y_2
Y_1
X_1 5 X_2 10 15 20 25 30 35 40 45 50 55
Time in hours

Table No: 11 Pharmacokinetic Parameter for Control

Pharmacokinetic Parameter	Values
K_E	0.0801 hr^{-1}
$t_{½}$	8.65 hrs
C_{max}	52.126 mcg/ml
t_{max}	24 hrs
$[AUMC]^{48}_{0}$	85375.412 mcg hr^2/ml
$[AUC]^{48}_{0}$	2711.62 mcg hr/ml
MRT	31.485 hrs

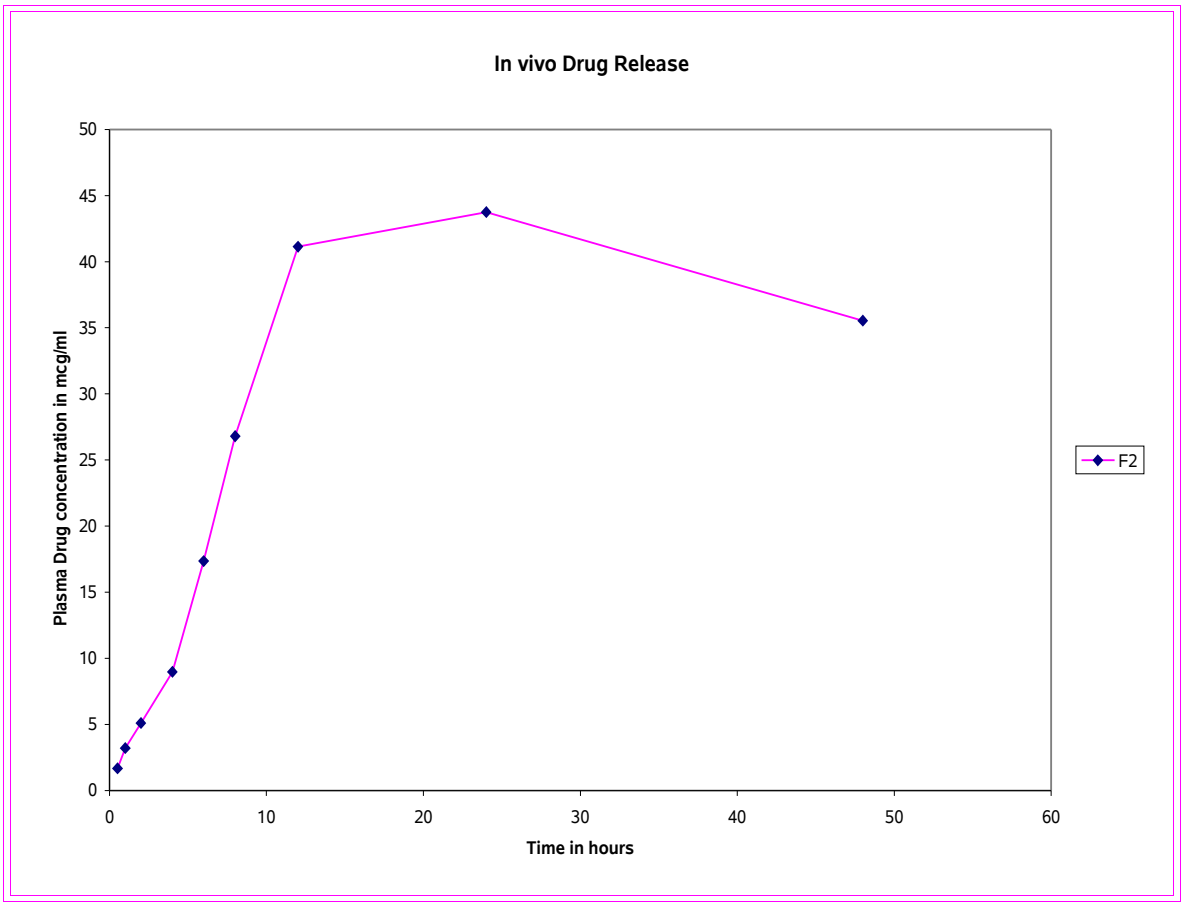
Table No.12 In Vivo Release Profile F2 Formulation

Time in hrs (t)	Absorbance at 365 nm	Plasma drug concentration µg / ml	Amount of drug in mg	% Drug release	C T	$[AUC]^t_0$ µghr/ml	$[AUMC]^t_0$ $\mu ghr^2/ml$
0	0	-	-	-	-	0.4187	0.2093
0.50	0.043	1.675	0.167	2.24	0.8375	1.2205	1.0111
1.00	0.060	3.207	0.320	4.29	3.207	4.153	6.7025
2.00	0.081	5.099	0.509	6.82	10.198	14.071	46.086
4.00	0.124	8.972	0.897	12.00	35.888	26.323	139.994
6.00	0.217	17.351	1.735	23.21	104.106	44.161	318.586
8.00	0.322	26.810	2.681	35.87	214.48	135.89	1416.2
12.00	0.481	41.135	4.113	55.04	493.62	509.292	9261.288
24.00	0.510	43.747	4.374	58.54	1049.928	951.552	33075.36
48.00	0.419	35.549	3.554	47.57	1706.352	0	0

∑ AUC = 1687.0812

∑ AUMC = 44405.0229

Figure No.17 In vivo Drug Release Profile for F2 Formulation



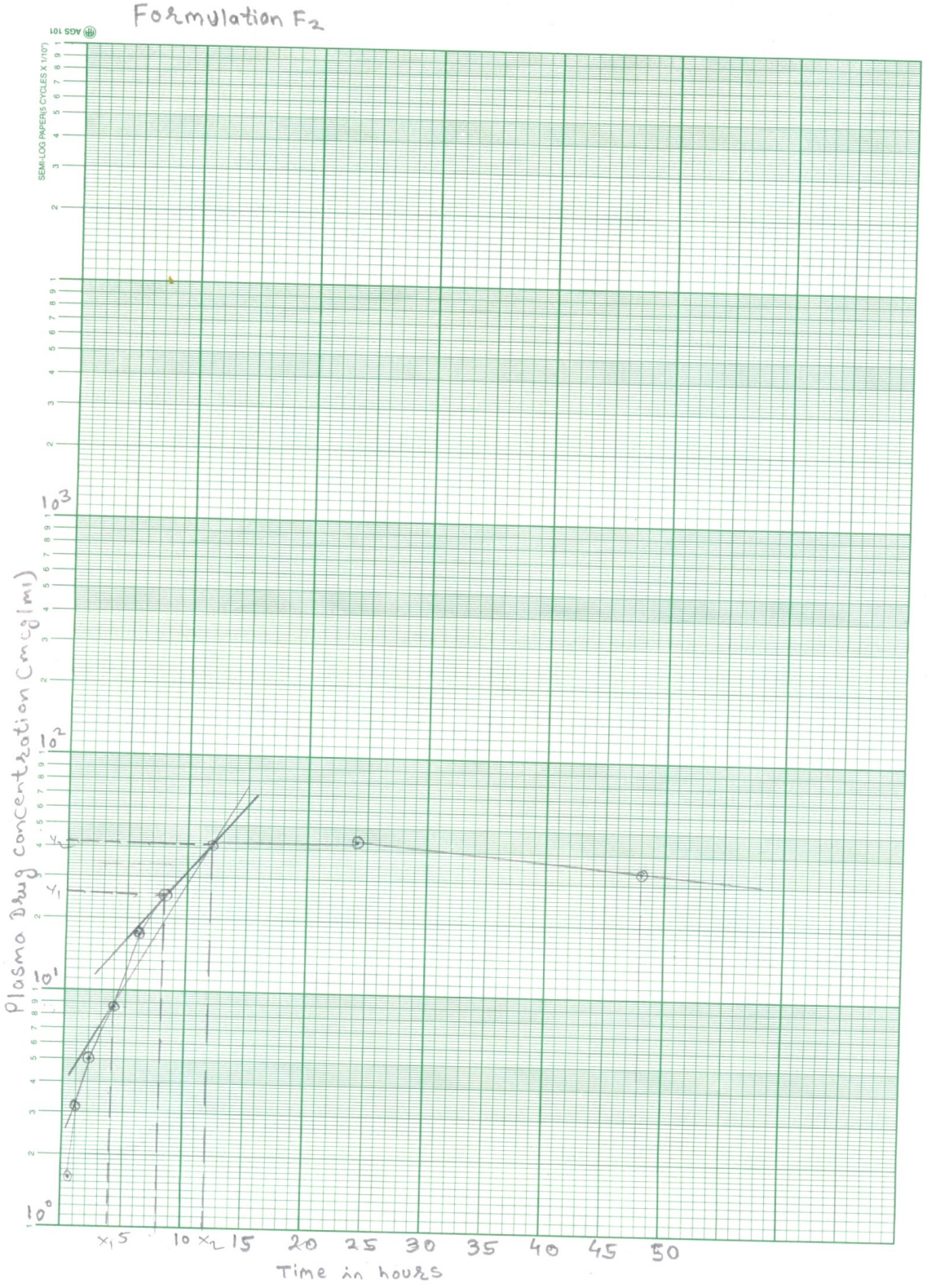

Formulation F2
SEMI-LOG PAPER 5 CYCLES X 1/10"
Plasma Drug concentration (mcg/ml)
Time in hours
10^3
10^2
10^1
10^0
10
15
20
25
30
35
40
45
50

Table No. 13 Pharmacokinetic Parameter for Formulation F_2

Pharmacokinetic Parameter	Values
K_E	0.1059 hr^{-1}
$t_{½}$	6.54 hrs
C_{max}	43.747 mcg/ml
t_{max}	24 hrs
$[AUMC]^{48}_{0}$	63887.720 mcg hr^2/ml
$[AUC]^{48}_{0}$	2022.765 mcg hr/ml
MRT	31.584 hrs

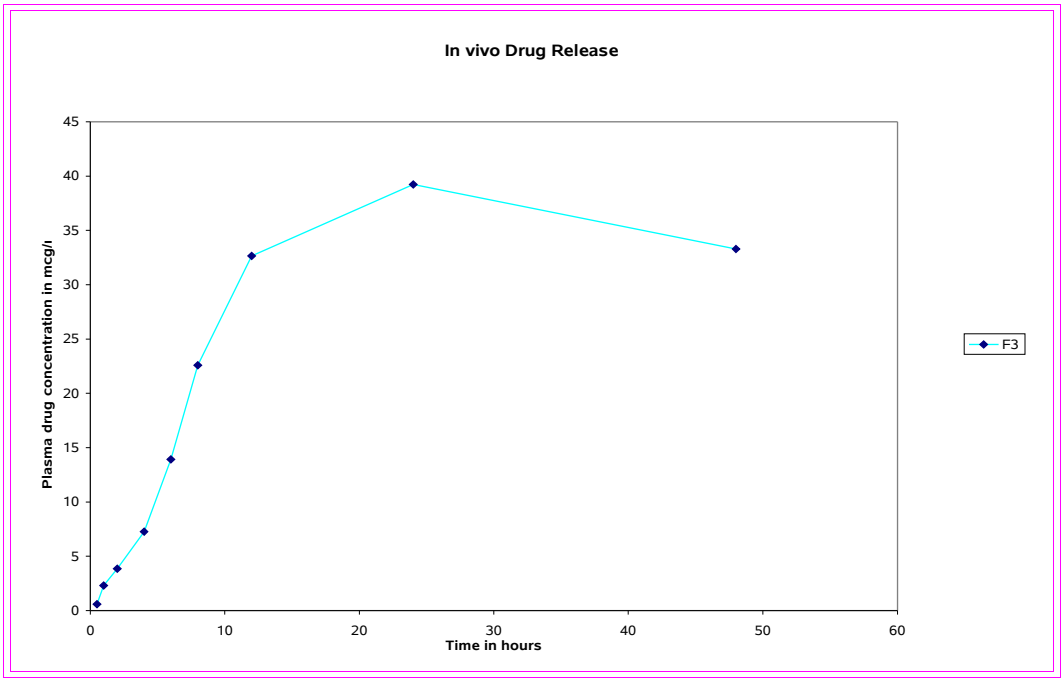
Table No.14 In Vivo Release Profile F3 Formulation

Time in hrs (t)	Absorbance at 365 nm	Plasma drug concentration µg / ml	Amount of drug in mg	% Drug release	C T	$[AUC]^t_0$ µghr/ml	$[AUMC]^t_0$ µghr^2/ml
0	0	-	-	-	-	0.1485	0.07425
0.50	0.031	0.0594	0.0594	0.703	0.297	0.725	0.6507
1.00	0.050	0.230	0.230	3.08	2.306	3.2215	4.99
2.00	0.067	3.383	0.383	5.13	7.674	7.4675	36.718
4.00	0.105	0.726	0.726	9.71	29.044	21.188	112.606
6.00	0.179	1.392	1.392	18.63	83.562	36.503	264.17
8.00	0.275	2.257	2.257	30.21	180.608	110.484	1145.2
12.00	0.387	3.266	3.266	43.71	391.992	431.454	8002.944
24.00	0.460	3.924	3.924	52.51	941.832	870.48	1270.044
48.00	0.394	3.329	3.329	44.55	1598.256	0	0

∑ AUC = 1481.67

∑ AUMC = 10837.3969

Figure No.18 In vivo Drug Release Profile for F3 Formulation



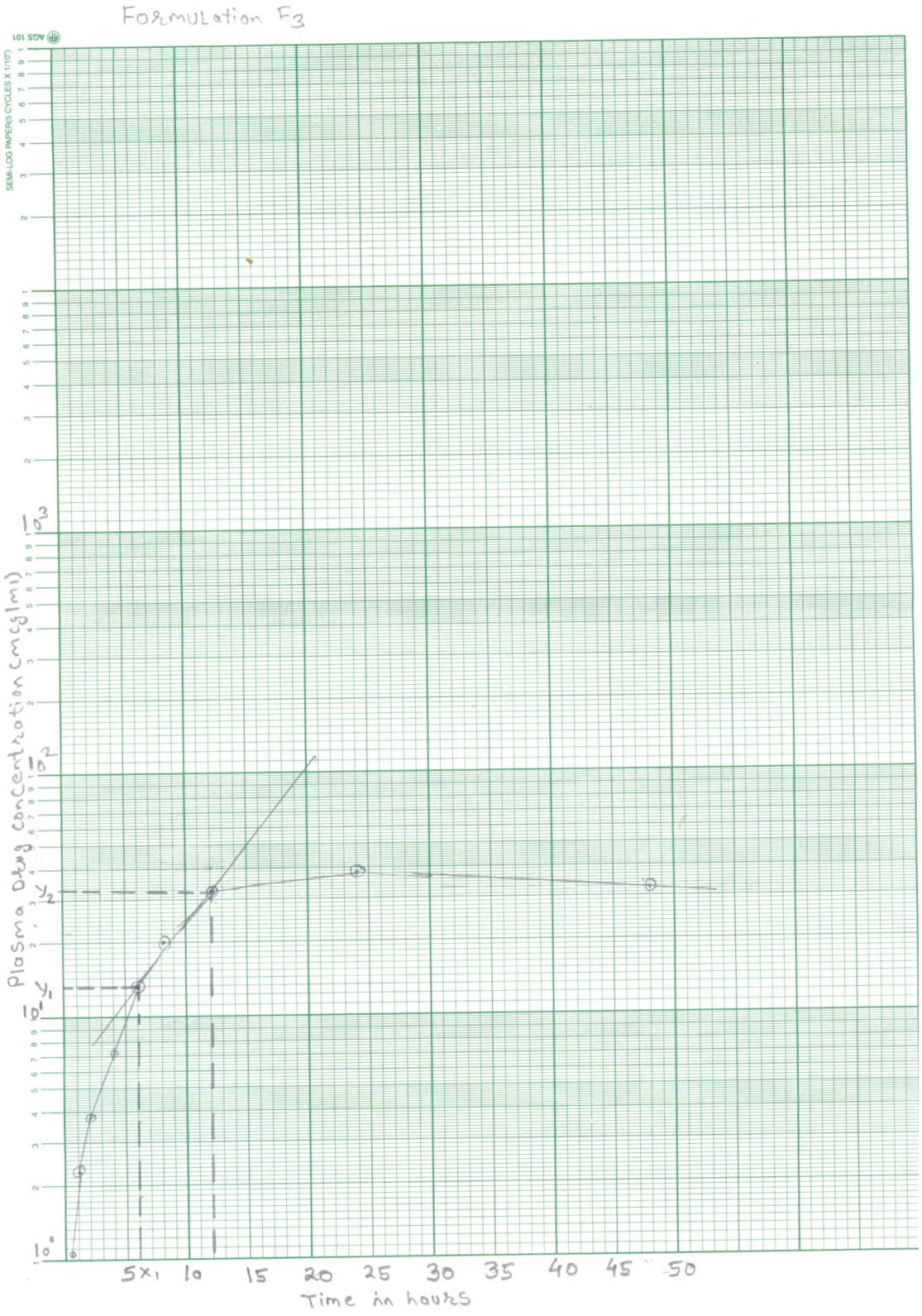

Formulation F3
SEMI-LOG PAPER(5 CYCLES X 1/10")
Plasma Drug concentration (mcg/ml)
10^3
10^2
Y_2
Y_1
10^1
10^0
5 X_1 10 15 20 25 30 35 40 45 50
Time in hours

Table No. 15 Pharmacokinetic Parameter for Formulation F_3

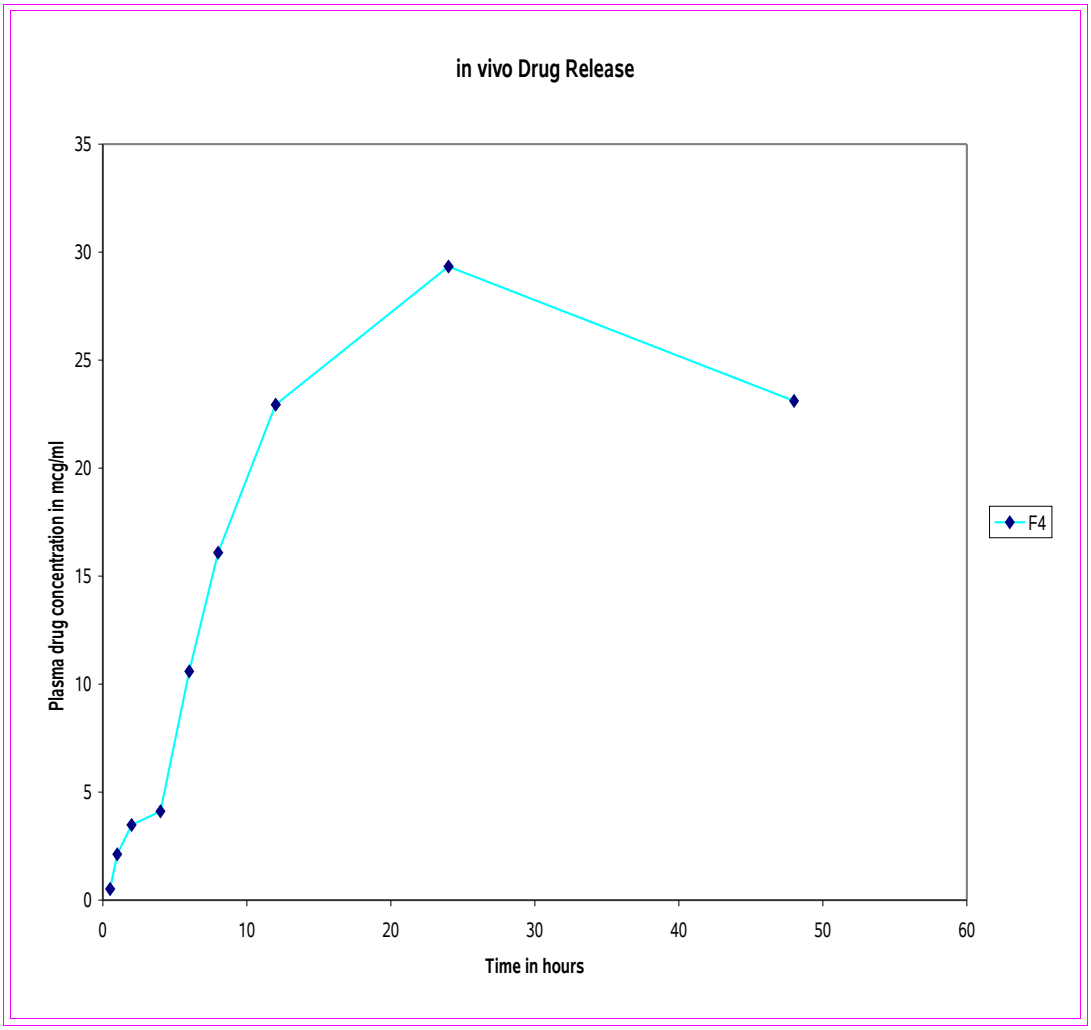
Pharmacokinetic Parameter	Values
K_E	0.09212 hr^{-1}
$t_{½}$	7.532 hrs
C_{max}	39.243 mcg/ml
t_{max}	24 hrs
$[AUMC]^{48}_{0}$	32119.593 mcg hr^2/ml
$[AUC]^{48}_{0}$	1843.200 mcg hr/ml
MRT	17.425 hrs

Table No.16 In Vivo Release Profile F4 Formulation

Time In Hrs (T)	Absorbance at 365 nm	Plasma Drug Concentration µg / Ml	Amount Of Drug In mg	% Drug Release	C T	$[Auc]^{t}_{0}$ µghr/ Ml	$[Aumc]^{t}_{0}$ $\mu ghr^{2}/Ml$
0	0	-	-	-	-	0.126	0.063
0.50	0.030	0.504	0.050	0.67	0.252	1.565	0.5945
1.00	0.048	2.126	0.212	2.84	2.126	2.8015	4.54
2.00	0.063	3.477	0.347	4.65	6.954	7.585	23.386
4.00	0.070	4.108	0.410	5.49	16.432	14.702	79.996
6.00	0.142	10.594	1.059	14.17	63.564	26.684	192.284
8.00	0.203	16.090	1.609	21.53	128.72	78.052	807.904
12.00	0.279	22.936	2.293	30.69	275.232	313.614	5875.344
24.00	0.350	29.333	2.933	39.25	703.992	629.4	21763.296
48.00	0.281	23.117	2.311	30.93	1109.616	0	60

∑ AUC =1074.5295
∑ AUMC = 28747.4075

Figure No.19 In vivo Drug Release Profile for F4 Formulation



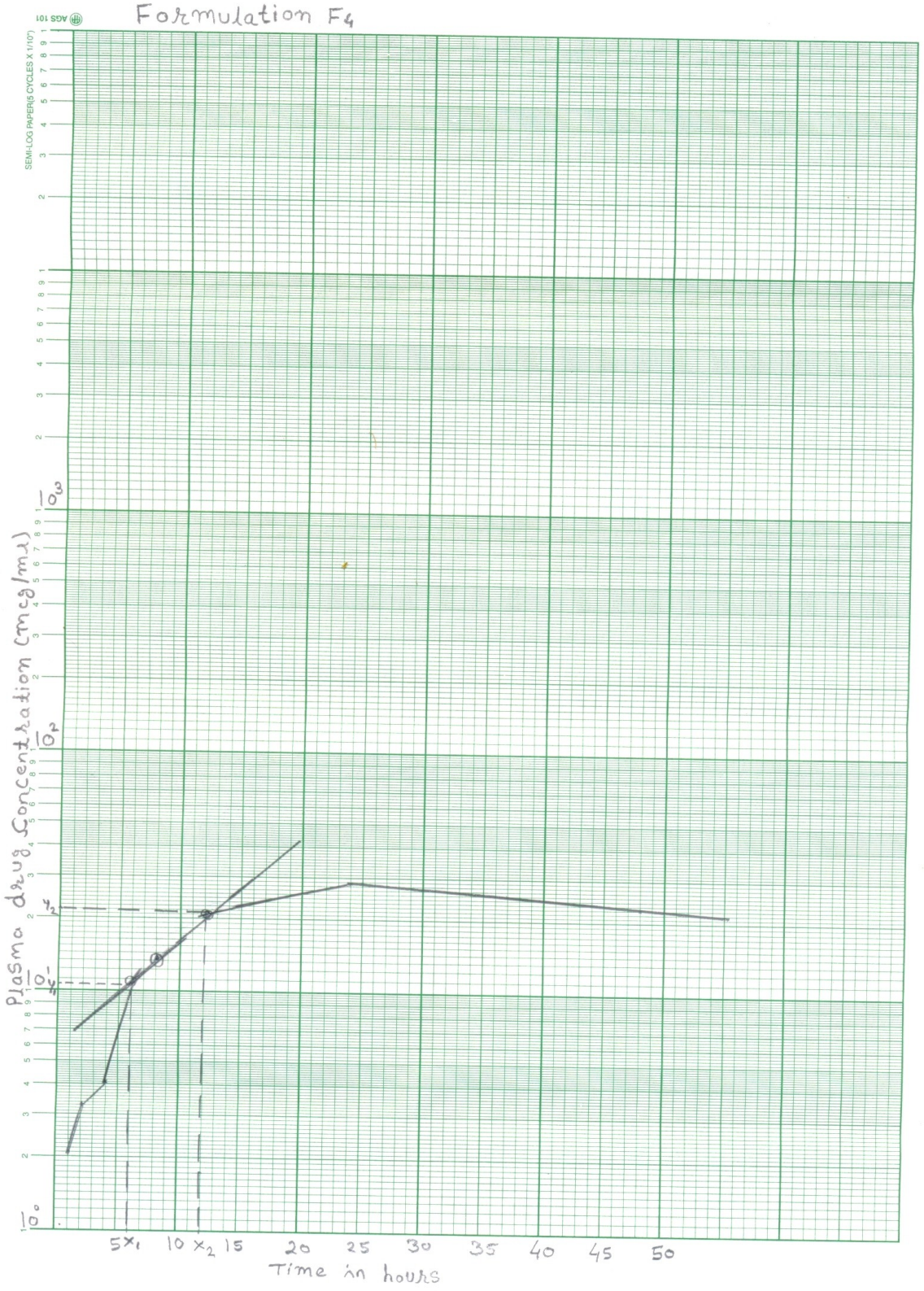
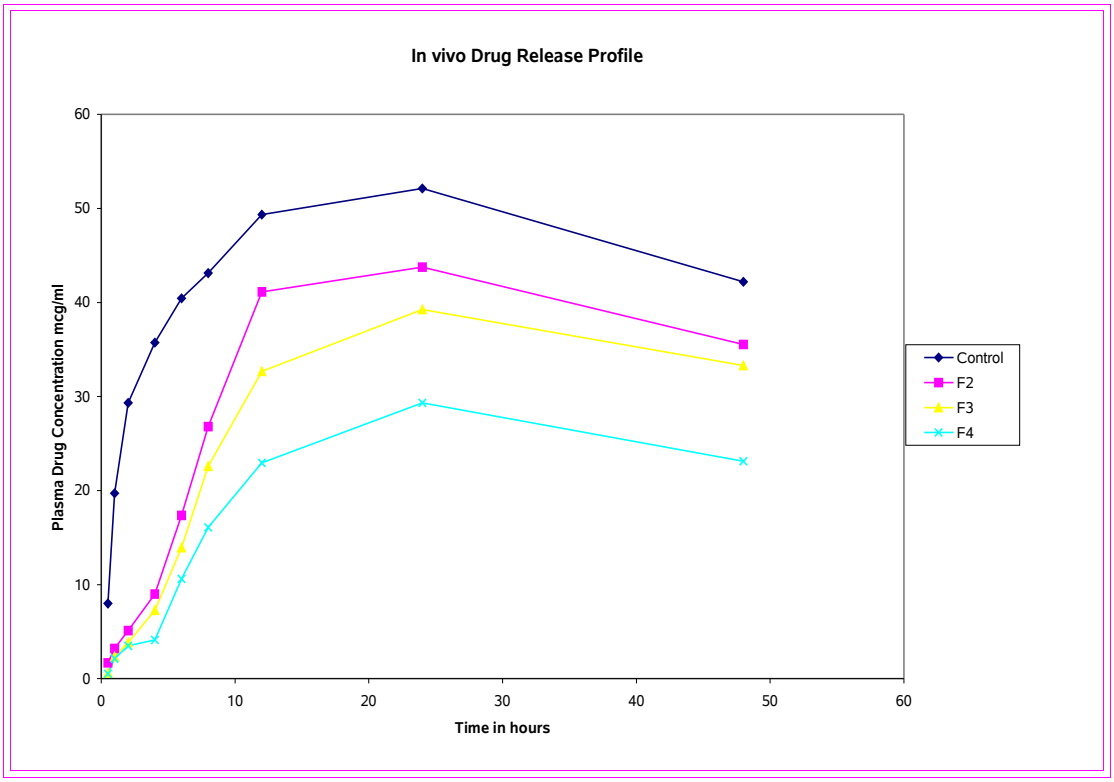

Formulation F4
SEMI-LOG PAPER(5 CYCLES X 1/10")
Plasma drug Concentration (mcg/ml)
Time in hours
5X1
10 X2 15
20
25
30
35
40
45
50

Table No. 17 Pharmacokinetic Parameter for F4 Formulation

Pharmacokinetic Parameter	Values
K_E	0.1287 hr^{-1}
$t_{½}$	5.384 hrs
C_{max}	29.333 mcg/ml
t_{max}	24 hrs
$[AUMC]^{48}_{0}$	38764.775 mcg hr^2/ml
$[AUC]^{48}_{0}$	1254.148 mcg hr/ml
MRT	30.90 hrs

Figure No. 20 Comparative In vivo Drug Release Profile



Skin Irritation Studies

The results of the skin irritation studies indicate that neither the adhesive nor Amlodipine have any noticeable irritation on the rabbit skin for the 48 hours study periods.

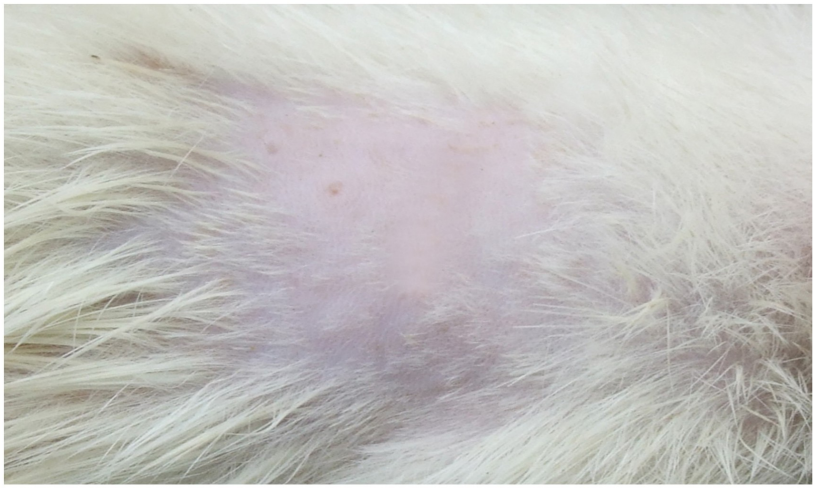
Stability Studies

Stability studies revealed that the drug content in formulation F_2, degraded respectively by 0.76%, 1.40% and 1.90% in a period of 90 days.

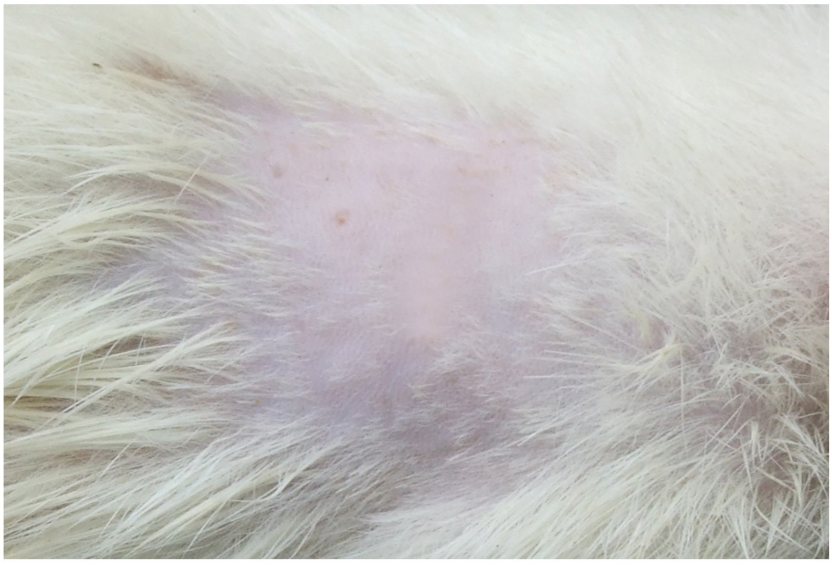
Table No.14 Stability studies for F2 Formulation

Time period in month	**Drug Content %**
Initial	99.24
1st Month	99.14
2nd Month	98.60
3rd Month	98.10

Before Skin Irritation Test



After Skin Irritation Test



8. SUMMARY AND CONCLUSION

The formulation of transdermal films were prepared using different ratio of the same polymers which assigned in increasing order according to permeation rates F_2>F_3> F_4. The results indicate that the films prepared with hydroxy proplymethyl cellulose as polymer of 1:4 (Drug: HPMC) F_2 was found to be best during in vitro study. Hence conclude that the increase in the concentration of polymer and film thickness decreases the drug release profile.

The in vivo studies demonstrate the transdermal drug delivery system gives sustained plasma concentration and avoids wide fluctuation of plasma concentration of amlodipine in rabbit.

- From the IR interpretation and UV- results the sample confirmed to be of amlodipine besylate.
- Melting point, partition coefficient, and solubility determined experimentally and molecular weight taken from literature.
- All physicochemical character support that the drug amlodipine may be suitable for transdermal drug delivery system.
- DSC studies carried out and it support the drug and excipient was compatible when they were in film form.
- In vitro studies carried out using F_2, F_3 and F_4 formulation as F_1 formulation polymer concentration not sufficient to form a film. F_2 formulation shows better release than F_3 and F_4.

In vivo studies carried out also using F_2, F_3 and F_4 and better release was shown only by F_2 formulation. The in vivo studies demonstrate the transdermal drug delivery system gives sustained plasma concentration and avoid wide fluctuation of plasma concentration of amlodipine in rabbits as diffusion of the drug was extended over a long period at control rate and it followed a pattern close to zero order release profile.

The results of the skin irritation studies indicate that neither the adhesive nor amlodipine have any noticeable irritation on the rabbit skin for the 48 hours study periods. Skin irritation and / or edema was not observed during skin irritation test. Hence, amlodipine films can be used in long term management of cardio vascular diseases, necessitating long term therapy by nullifying the common adversities of the drug.

Stability studies revealed that the drug content in formulation F_2 degraded respectively by 0.76%, 1.40% and 1.90% in a period of 90 days.

Hence, finally conclude that the study shows the feasibility of formulating rate controlled transdermal drug delivery system for amlodipine besylate in order to achieve improved bioavailability and nullifying the common adversities of the drug.

BIBLIOGRAPHY

1. **Christopher T. Rhodes,** Modern pharmaceutics, second edition, Marcel Dekker ,INC NewYork, P 635-668.

2. **Chien, YIE.W. ,** Novel Drug delivery system, Marcel Dekker , INC , Newyork , P1-6.

3. **Joseph R. Robinson and Vincent H.L. Lee,** Controlled drug delivery, second edition, Mercel Dekker, INC, Newyork ,P1-6.

4. **Michael E.Aulton.,** Pharmaceutics, The Science of dosage form design, ELBS, P381-400.

5. **Remington:** The Science and Practice of Pharmacy, 19th edition, Mack publishing company, Pennsylvania P1600-1675.

6. **Praveen Tyle.,** specialized drug delivery system Vol.26, Mercel Dekker, INC P 3-13

7. **Dr. P.K Chaudhary., et al.,** Transdermal drug delivery system: An overview, The Eastern Pharmacist, 45-47, July-97.

8. **S.C. Basak., et al,** Transdermal drug delivery system: An overview, The Eastern pharmacist, 63-67, Aug'97.

9. **Dr.Krishna., et al.,** Transdermal Drug Delivery System: An overview, The Eastern Pharmacist, 17-22, Oct – 1991.

10. **Jawahar N., et al.,** Evaluation of different Formulation variables on Transdermal films of verapamil hydrochloride: The Indian Pharmacist P.67-69, June –2007.

11. **Johnson M.E,et al.,** A mechanism to enhance transdermal drug delivery. J.Pharm sci., 85, 670-679, 1996.

12.www.ahfs drug information .com

13. *www.szhaveri.com*

14. **V.H.Kulkarni et at.,** Trans dermal delivery of Terbutaline Sulphate through modified chitosan membrane : Indian J.Pharm.Educ.38(4) Oct – Dec 2004 P.189.190

15. **D.Monti. et al.,** Comparison of the effect of ultrasound and of chemical enhancers on trans dermal permeation of caffeine and morphine through hairless mouse skin in vitro: International J. of pharmaceatics:229 (2001) P.131-137

16.www.elsevier.com

17. International Journal of Pharmaceutics 219 (2001) P.161-176

18. **N.K. Jain et al.,** Advances in Transdermal drug delivery system. Pharma Times:Vol.32,5,21-25, May 2000

19. **Gerard J.Tortora, Sandra Reynolds Grabowski,** Principles of anatomy and physiology, **Johan wileyand sons,** INC.9th edition, P.140 – 143, 2000.

20. **Montagna, W.andParakkal, P.F,** The structure and function of skin. Academic press New York, 8-10, 1974.

21. **Elias, P.M.** Lipids and the epidermal permeability barrier, Arch.Dermatol, 95, 1981.

22. International Journal of Pharmaceutics 224 (2001) 1-18.

23. **J.Y.Park,et,al.,** Comparative pharmacokinetic and pharmacodynamic characteristics of amlodipine besylate and amlodipine nicotinate in healthy subject, Int.J.of clinical pharmalogy and Therapeutics, vol.44-No.12/2006 (641 – 647)

24. **Richard S. Morosco.,et al.,** Stability of Amlodipine Besylate in two liquid dosage forms; Journal of the American Pharmceutical Association Vol.39, No.3 May/June 1999.

25.www.Pubmed .com

26. *http://en.wikipedia.org*

27. **James swarbrick.,etal.,** Encyclopedia of Pharmaceutical Technology.Vol.l.Mercel Dekkar, INC, P. 1-30.

28. **Guyton C.,** The text book of medical physiology, 8th Edition, Marcel Dekkar, P888-890

29. **Cornwell, P.A & Barry, B.W.,** J.Pharm. Pharmacol., 43,56,1991

30. **Wong O and T Suzuki N,** Int.J.Pharm. 52 191 –201, 1989.

31. **Kraemer BK., et al.,** European J.Clin.Pharm. 43, 85-88, 1992.

32. **Smith, E.W.Maibach, H.I.,** Percutaneous Penetration enhancers CRC Press, Boca Ration, 1995.

33. **Stanely S.David, Lisbeth illum.,** Polymers in controlled drug delivery. Wright, Bristol. P1-15 .

34. **Dr.N.Udupa**., Process in drug delivery research Manipal Experience, P75 –162.

35. **Barrie C. Finnin and Timothy M.Morgan** Transdermal penetration enhancers. Application, Limitation & Potential. J. pharm.Sci. vol. 88, no 10 955-958-1999

36. **Churchill Livingstone,** Therapeutic drug delivery vol.1, P 75-79.

37. *www.medscape.com*

38.www.drugs.com

39.www.medicincenet.com

40. **White W. B., et al.,** Journal of Hypertension I (suppl.4): 575-580, 1993.

41. **Lahiri A., et al.,** American Journal of cardiology 56. 769-775.1987.

42. **Kaidi Zhao., et al.,** Mechanism of in vitro percutaneous absorption

43. Enhancement of Tamoxifen by enhancers J. Pharm. Sci, 83, 4,552-555,1994.

44. **Cheon Koo Lee, et al.,** Skin permeability of various drugs with different lipophilicity. J. Pharm.Sci, 83, 4,552-55, 1994.

45. **Janicki.S. et al.,** In vitro penetration of isosorbide dinitrite from a tansdermal device into rat skin pharmazie 48,865 (Nov) 1993

46. **S.Narasimha Murthy et al**., Formulation and evaluation of transdermal films of Terbutaline sulphate. Indian. J. Pharm .Sci. 5715, 207-209, 1995.

47. **N.Udupa., et al,.** Evaluation of piroxicam oral and Transdermal preparation: Indian. J. Pharm.Sci.67(5),152-154,1991

48. **Chien Y.W et al.,** Transdermal Controlled administration of indomethacin Pharm Res. 5,103-106, 1998.

49. **Ajay Gupta et al.,** Gelatin based Transdermal therapeutic system. Indian drugs,29(7),309, 1992.

50. **P.R.P.Verma and SunilS.Iyer et al.,** Controlled transdermal delivery of propranolol using HPMC matrices: Design and in vitro and in- vivo evaluation.J. pharm.Pharmacol,52,151-156,2000

51. **Leon Lachman., et al.,** The Theory and Practice of Industrial Pharmacy, 3rd edition, Varghese publishing House, Bombay, P.440-460.

52. **Martindale's** Extra Pharmacopoeia 30th edition, The Pharmaceutical press, London, 1996, P840.

53. **Kusum devi and K.L.K Paranjothy.,** A mathematical model to determine feasibility of drug delivery through transdermal route. Int .J.Pharm.Sci., 67-69, Jan-Feb-2000.

54. **Milo Gibaldi.,** Biopharmaceutics & clinical pharmacokinetics Lea Febiger, London, 1991. PP 146-176t.

55. **Shobha Rani R.Hiremath**., Textbook of Biopharmaceutics & Pharmacokinetics. Prism Books Pvt. Ltd; Bangalore, PP 83-100.

56. **S.K.Kulkarni.,** Hand book of Experimental pharmacology 3rd edition. 164-165,1999..

57. British Pharmacopoeia, Vol. I : 2005, P.no : 126-127

58. **Clarke's** Analysis of drug and poison, Third edition, P.no: 629.

59. Identification of drug in Pharmaceutical formulation by Thin layer chromatography, second edition by **P.D. sethi / Dilip charegaonkar**; P.no: 79.

60. Indian Pharmacopoeia – 1996, Vol –II (P-Z), P. A – 144.

61. Drug delivery system, second edition by **vasanth V.Ranade** and **Mannered A. Hollinger** P. 207 – 248, 63-114.

62. **Sahu R. & Patel V.B**. Indian drug 43 (2) Feb 2006, P.no : 160-161

63. **Gaud R.S, et.al.,** Indian drug 43(3) march – 2006 ; P.no : 245-251.

64. **Loganathan V, Senthil kumar,** Indian drug 40 (11) November – 2003,P.No: 661-664.

65. **V. Sankar, D.Benito johnson,** Indian J. Pharm. Sci., 2003, 65 (5) : 510-515.

66. **Kalia YN, Guy RH. et.al.,** Interaction between penetration enhancer and lontophoresis : effect of human skin impedance in - vivo. Controlled release. 44, 32 -42, 1997.

67. **Vanvbever, R,** Transdermal delivery of Metoprolol by electroporation. Pharm. Res. 11, 1657- 1662, 1994.

68. **Jeffrey T. Cantrell, et.al.,** Transdermal Extraction of interstitial Fluid by low frequency Ultrasound quantified with $^{3}H_2O$ as a tracer molecule. J.Pharm.Sci., vol 89, 9, 1170 -1178, 2000.

69. **Beckett and Stenlack ,** Text book of Pharmaceutical analysis Vol. I Pl 08 -112.

70. **Willard, et.al.,** Instrumental methods of analysis, sixth edition, CBS, publication, India, P 177-216.

71. **J.Y.Park .et al.,** International Journal of Clinical Pharmacology and Therapeutics, Vol.44-No.12/2006 (641 –647).

72. **Paul W. stott. et.al..,** International Journal of Pharmaceutics 219 **(**2001)161 – 176.

73. **D.Monti. et.al.,** International Journal of Pharmaceutics 229 (2001) 131 –137.

74. **Rajagopal K.et.al.,** The Indian Pharmacist Vol. IV, NO. 31, Jan – 2005 P.No:77 –80.

75. **L.M.A Nolon. et.al.,** International Journal of Pharmaceutics 257(2003) 41-55.

76. **M.D.Blanco.et.al.,** International Journal of Pharmaceutics 255(2003) 99 -107

77. **A.Nokhodchi.et.al.,** International Journal of Pharmaceutics 250 (2003) 359 –369.

78. **Xiaohong Qi et.al.,** International Journal of Pharmaceutics 259 (2003)39-45.

79. **P.RamaRao.et.al.,** European Journal of pharmaceutics and Biopharmaceutics 56
(2003) 81-85

80. **Sandip B.Tiwari – et. al.,** International Journal of Pharmaceutics 260(2003)93-103.

81. **Michael A.Ashburn.et.al.,** The Journal of pain, Vol.4, No6 (August)2003, PP291-297

82. **Jagdish Singh. et. al.,** International Journal of Pharmaceutics 263 (2003) 25- 33.

83. **Franziska Grafe. et.al.,** European Journal of pharmaceutical sciences 21 (2004)309-312.

84. **P.N.Kotiyan.et.al;** International Journal of Pharmaceutics 270 (2004) 47 –54.

85. **Elvira Fscribano.et.al.,** European Journal of Pharmaceutical Sciences 19 (2003) 203-210

86. **Silvia Latsch, Jorg kreuter .et.al.,** European Journal of Pharmaceutics and Biopharmaceutics 56 (2003) 43 –52.

87. **M.Jayne Lawrence .et.al;** International journal of pharmaceutics 250 (2003) 65 –83.

88. **Akira yamamoto.et.al.,** International Journal of Pharmaceutical Sciences 18 (2003) 63 –70

89. **Ramesh panchagnula. et.al;** European Journal of Pharmaceutical, sciences 18 (2003) 71-79.

90. **David J. Mazzo,** International Stability Testing, 14 – 24.